Variation within and among populations of the endangered Cape Verde warbler: implications for conservation

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A thesis submitted for the degree of Doctor of Philosophy

School of Biological Sciences

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March 2017

This thesis is dedicated to Cornelis J. Hazevoet, who did so much for ornithology in Cape Verde and for the Cape Verde warbler in particular.

Abstract

In this thesis, I investigate variation among the three remaining populations of the Cape Verde warbler *Acrocephalus brevipennis,* with the aim of defining conservation units and thus informing conservation. I used neutral molecular markers to assess genetic diversity and infer the evolutionary history and adaptive potential of the populations. I found a gradient of diversity from the largest population of Santiago to the smallest one of S. Nicolau, which has been isolated from the populations of Santiago and Fogo for longer than those have been isolated from each other. I measured differences in male song between the populations and whether individuals behaved differently when exposed to songs from the other populations, to determine if song might play a role in reproductive isolation. While there is high intra-population variability in male song, birds do not react differently to songs of males from other populations. I then investigated which habitat traits determined the presence of the bird and whether this differed between the three islands. Evergreen dense vegetation cover predicted the wider areas occupied by the warbler. Structural vegetation traits did not differ between sites used on the islands, but plant species composition did. Finally, I assessed morphological differences between the three populations. I found that males on S. Nicolau have shorter tarsi and longer, narrower bills. These morphological differences match the pattern of neutral genetic divergence, suggesting that the role of drift in driving these differences has not been overridden by selection. Collectively, my results show small but significant divergence between the three populations, particularly between S. Nicolau and the two other populations. This divergence has implications for this bird's conservation, as the differences observed warrant considering the populations as different management units.

Acknowledgements

This PhD was a great adventure, both professionally and personally. It would not have been completed without the help of a huge number of people who gave their whole-hearted contribution at specific times.

First of all I would like to thank Sara Pardal for sending me that email, back in 2012, telling me about a researcher at the University of East Anglia who was looking for a student to do a PhD on the Cape Verde warbler. Sara, if it weren't for you, I would have never known about this opportunity and I would have missed what was possibly the most exciting job I had in my life. I am incredibly grateful to you!

Secondly I would like to thank my excellent supervisory team: David Richardson, Iain Barr and Nigel Collar. David, you have been a great inspiration and have made me a better person and a better professional. Thank you for your persistence, determination, optimism and support. Iain, thank you for your enthusiasm, great ideas and constant support. Nigel, thank you for always finding time to answer my questions and soothing my anxiety, even though you are an incredibly busy man. All of you are really intelligent, have excellent work ethics and are incredibly professional, which contributed enormously to make this PhD thesis the best possible one.

To my friend and colleague David J. Wright, what would have I done without you? You contributed officially to the chapter on genetic diversity, and unofficially to all the other chapters because you helped me with R, you reviewed a lot of my drafts and helped me made sense of incipient ideas. Thank you for your support, your optimism, for having attention to detail, and for always having time for me and an opinion about my work.

Paul Donald, thank you for your help with trying to find this little brown bird on S. Nicolau and for suggesting and providing guidance on the the MaxEnt analysis that we used in the habitat chapter. Ricardo Jorge Lopes and Raquel Vasconcelos, thank you for your help and guidance in organising fieldwork and input on the genetics chapter. Gonçalo Cardoso, thank you so much for your help with song analysis and statistics, and for always trying to answer my every question. Sérgio Timóteo, thank you for always taking the time to answer my stats questions. Eduardo Garcia-del-Rey, thank you for putting me in touch with the Museum of Oslo and for allowing me to contribute to your book on the Cape Verde birds. Juan Carlos Illera, Martim Melo, Martin Haase, the University of Oslo Natural History Museum and the Seychelles Warbler Research Group for extra CVW samples; Silke Fregin, Urban Olsson, Juan Carlos Illera, Lewis Spurgin for input on molecular work for the genetics chapter.

Cornelis J. Hazevoet, the person who most studied this bird before we did, thank you for all our meetings at Cister, where we discussed at length the Cape Verde warbler and how it is to live and work in Cape Verde, and for all the emails and papers you sent.

Tommy Melo from Biosfera I and Pedro Geraldes from SPEA, thank you so much for helping me get started with my fieldwork in Cape Verde, for getting the Cape Verdean ringing scheme going, for all the advice and for finding me a place to stay – and Pedro, in your case, for letting us stay at your place in Mindelo when you were not there!

Aline Rendall, Alexandre Nevsky Rodrigues, João Mascarenhas, Lindaci Oliveira and Emitério Ramos, thank you very much for supporting this project and for helping with everything you could during our fieldwork, especially for finding us places to stay that we could afford with our limited budget! Without your help, we would have been unable to do this much work in the field and this PhD would have not been finished! Also a big thank you to all the Cape Verdean biologists who helped us during fieldwork: Gilson Semedo, Evaldino Pereira, Gabriel Tavares, Herculano Dinis, Maria "Chia" Auxiliadora do Nascimento, Nelson Santana, Franciscana Sequeira, Ivani Duarte, Gilda Monteiro and others. Thank you for your interest, for your help in finding the birds, especially on S. Nicolau, for setting the mist-nets, for your advice and for your friendship and enthusiasm!

Celestino Duarte, this work would have been severely impaired without your help. Thank you for letting us stay at your studio for three weeks when we first arrived in Cape Verde, for finding us and renting us that big black jeep, and for always trying to help with bureaucracy.

I would like to send special thanks to the fantastic people of Mosteiros, Pai António and Monte Barro, Fogo: Ilda, Fidjim, Nene, Manu, Tony, Rui Fontes, Batinzim, Dona Christine, and so many others. You made us feel home during our time there, made our stay there much cheaper and therefore longer! Special thanks to Nene for showing us all those Cape Verde warbler territories. Also special thanks to Xexe Kifra, our "Christmas miracle", the man who was on his balcony when we stopped at the ATM machine on our way to Chã das Caldeiras on Christmas day. Thanks to you, our fieldwork group got to spend our Christmas evening on that magical place!

Now the part that always brings a silly tear to my eye: thank you very much to my incredible, wonderful, fantastic field assistants! It was a great pleasure to work with you, thank you for putting up with me, for always doing your best and for your friendship. Jaelsa Moreira and Naya Sena, my Cape Verde field assistants, two amazing women and good friends, you will always be in my heart! Jaelsa, thank you for your positive and rational spirit, for giving this project your best, for being a good friend and for partying with me on Brava! Naya, I have no words, you are such an inspiring woman and great friend, professional, curious and motivated, and always helped me in every possible way, whether it was to find us a place to stay or an extra field assistant. Thank you to Naya's mum Maria dos Reis "Nunui" for letting us stay at your place for such a long time when we had to work on Santiago, for your inspiring calm and wise words. Gilson Montrond, I only met you during the last field season but you were also great, with your eagle eye that never missed one bird and your tree-climbing abilities. Andrew Power, my first field assistant ever, thank you for meeting me at the airport of Praia at 2am when neither of us knew what was going to happen, for putting up with my very emotional this-is-amazing mood during two months (Torbjörn did not suffer that much haha), for always doing your best and contributing in every way possible to the success of this project. Josh Jenkins Shaw, thank you for your help and professionalism, especially on Fogo and with ageing the birds. Torbjörn Blixt aka "Golden Magnus" thank you for coming with me everywhere, even when we took that hike on Santo Antão on your birthday and did not find anything to eat (only two coca colas to drink) until night time! Thank you for helping define the methodology for the habitat chapter, for being professional, accurate, reliable and always in a good mood, even when you got sea sick, and for making us laugh by astonishing all the villagers who had never seen such long hair and so many tattoos! And finally Jeroen Arnoys who came to help twice, thank you for doing everything you could to make this project a success, from setting mist-nets to buying that thank you gift for Nunui.

Thank you to all the lovely people here at BIO and especially David Richardson's lab members, past and present, for being supportive and collaborative. Special thanks to Ellie Fairfield for helping me with lab work; to Cat Morrison for helping with my stats questions; to José Alves who is not here anymore but helped me get started and was always so positive; Dave Wright I mentioned already, as he deserves a special place; and finally thanks to Elizabeth Duxbury for being such a lovely office mate, for keeping me company during our late-night work marathons and for her contagious laughter!

Thank you to all my friends here in Norwich, for being so lovely, I am very lucky to have met you. The closest ones Isabel Ponce, Fiona Joyce, Aiko Patmore, MariaBella Bell, Paolo Grazzini, Rute Portugal, Laura Campeão and the whole salsa family, thank you for everything from being there for me to laughing with me (and at me) and for cooking food for me when I was living off junk food! While you did not contribute directly to this work, you contributed enormously to my wellbeing and happiness. I will always remember you guys with a big smile!

Now the people who were there for me even though they were far away: thanks to all my friends, back home and far away in other countries, for always believing in me. A very special thanks to Marta Francisco for being a true friend in every sense of the word, for encouraging,

supporting and understanding me from so far away, for being serious, focused and reliable, you are a true rock! But a beautiful one! Thank you to my mum and dad for everything they did for me their whole lives, but specially to my mum for helping me with a million bureaucracies which come with doing a PhD in one country while funded by another country.

Last but not least, thank you to all the funding organisations that contributed to this project: Fundação para a Ciência e Tecnologia, University of East Anglia, BirdLife International, The Rufford Foundation, The Royal Society for the Protection of Birds, African Bird Club and The A. G. Leventis Foundation (and Phil and Carolyn Hall for mentioning it to me).

Ok, I hope I did not forget anybody…! If I did, it's because I'm getting old…!

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Author Contributions

At the time of final submission, one of the data chapters presented in this thesis has been published. I am lead author on that manuscript and I have made by far the largest contribution to the work presented in this thesis. Below, I provide a citation for each data chapter, highlight authorship and specify my contributions.

Chapter 2: Batalha HR, Wright DJ, Barr I, Collar NJ, Richardson DS (2017) Genetic diversity and divergence in the endangered Cape Verde warbler *Acrocephalus brevipennis*. *Conservation Genetics*, **18**, 343-357.

- HRB organised and undertook the fieldwork, did the molecular work and analysis and drafted manuscript (70%).

Chapter 3: Batalha HR, Barr I, Collar NJ, Richardson DS (in prep) Diversity and divergence in song in the three populations of Cape Verde warbler *Acrocephalus brevipennis*

- HRB designed the field methodology, organised and undertook the fieldwork, collected and analysed all the data and drafted the manuscript (75%).

Chapter 4: Batalha HR, Barr I, Collar NJ, Richardson DS (in prep) Habitat preferences in the Cape Verde warbler Acrocephalus brevipennis and implications for conservation

- HRB designed the field methodology, organised and undertook the fieldwork, collected and analysed all the data and drafted the manuscript (75%).

Chapter 5: Batalha HR, Barr I, Collar NJ, Richardson DS (in prep) Morphological variation in the Cape Verde warbler *Acrocephalus brevipennis*

- HRB organised and undertook the fieldwork, analysed all the data and drafted the manuscript (75%).

Appendix I: Batalha HR (unpublished) CONSERVAÇÃO, ECOLOGIA E GENÉTICA DA FELOSA DE CABO VERDE *Acrocephalus brevipennis* | CONSERVATION, ECOLOGY AND GENETICS OF THE CAPE VERDE WARBLER *Acrocephalus brevipennis*

- HRB networked with local authorities, obtained permits, managed assistants, collected data, and outlined, drafted and translated the report (90%).

Chapter 1

General introduction

Habitat of the Cape Verde warbler Acrocephalus brevipennis *in São Jorge dos Órgãos, Santiago, November 2013.*

1.1 Global conservation challenges

"In the end we will conserve only what we love; we will love only what we understand; and we will understand only what we are taught."

Baba Dioum, 1968

Over the last few centuries, a large proportion of global biodiversity has been lost, with once common species becoming rare or extinct due to anthropogenic impacts (Baillie *et al.* 2004; Butchart *et al.* 2010; Ceballos *et al.* 2015). These impacts include, but are not limited to, habitat destruction, the introduction of exotic plant species, pathogens and predators, pollution and climate change (Butchart *et al.* 2010; Mooney & Cleland 2001; Sax & Gaines 2008). Currently, these threats continue to cause extensive biodiversity declines (Baillie *et al.* 2004; Butchart *et al.* 2010). Conservationists have fought hard to reverse this problem (Baillie *et al.* 2004), but for socio-economic reasons, the time and financial resources that can be allocated to conservation are scarce (Brooks *et al.* 2006; Myers *et al.* 2000). Consequently it is crucial to assess the key factors or issues related to the threats to biodiversity and identify conservation priorities in terms of where and how we handle them (Caughley 1994; Myers *et al.* 2000). Defining conservation priorities is not always straightforward (Fraser & Bernatchez 2001; Moritz 1994a), but the first step is to gather enough information about vulnerable species and ecosystems for proper conservation assessments to be made (IUCN 1995). For species, this often involves clarifying the evolutionary or taxonomic status of populations and the relationship between them, characterising genetic and phenotypic diversity within populations, and understanding how habitat preferences may differ between them (Allendorf & Luikart 2007; Frankham *et al.* 2009; Groom *et al.* 2006; Primarck 2012).

1.2 Population divergence

The classification of living organisms into the Linnaean taxonomic ranking system is fundamental for the study of ecology, conservation and evolution (De Queiroz & Weins 2007; Tobias *et al.* 2010). It is a step towards cataloguing life on Earth and thus forms an important basis for applied decision making. When applied to conservation planning, the species concept is a fundamental measure of biodiversity richness for policy makers which allows the design and monitoring of targeted conservation actions (Baillie *et al.* 2004; Tobias *et al.* 2010). The biological species concept states that a species is a group of interbreeding individuals which are reproductively isolated from other individuals (Dobzhansky 1937; Mayr 1942). However, defining species and related intra specific taxa can be complicated for several reasons (Hiers *et al.* 2016; Ryder 1986). One is that natural populations exist along a continuum of gradual temporal divergence, which can range from metapopulations to species complexes (Marske *et al.* 2013; Peccoud *et al.* 2009; Shaw & Mullen 2014). An additional problem is that, for allopatric populations, it is often not possible to test for reproductive isolation in the wild, and this makes the status of many geographically isolated populations uncertain (De Queiroz & Weins 2007; Edwards *et al.* 2005; Grant *et al.* 2000; Orr & Smith 1998). Therefore, other species concepts were created, particularly for well-studied taxa such as the vertebrates. One of the most popular is the phylogenetic species concept, which states that a species is the smallest possible group of individuals that share the same common ancestor (Cracraft 1983; Zink 2006). This implies that species are reciprocally monophyletic, i.e. all lineages within each group share more-recent common ancestors than lineages from distinct groups (Avise 2000). As this often involves examining genetic markers within and among populations, the problem of which loci to examine arises (Price 2008). Different genetic loci have different transmission modes and mutation rates and can exhibit differential recombination, incomplete lineage sorting and hybridisation, thus it becomes difficult to select which parts of the genome to use (Avise 2000; Avise 2004; Coyne & Orr 2004). Furthermore, basing species classification on genetic divergence alone may be unwise (Price 2008; Tobias *et al.* 2010). Recently, the scientific community has come to consensus that one diagnostic trait is not enough to classify populations as species (Tobias *et al.* 2010), and that several traits should be used in conjunction, for example genetics, morphology and ecology (Helbig *et al.* 2002; Irwin *et al.* 2001; Toews & Irwin 2008). However the use of multiple traits can sometimes complicate the problem, as there is often discordance between genes and other population traits, such as ecology or morphology (Phillimore *et al.* 2008; Walsh *et al.* 2017).

Divergence in easily observable and measurable traits, such as morphological or behavioural traits, has long been used to categorise and classify populations into different subspecies or races (Brower 1994; Cramp & Perrins 1992; Mallet *et al.* 1998; Thomas 1926). Morphological traits can include colouration, size, shape and form (the relative positions of different body parts of animals and plant structures), and differences in these traits can be caused by different evolutionary history or local adaptation (Benkman & Miller 1996; Grant 1985; Odum 1971). Such traits were once widely used to infer relationships between populations and species, but with advances in molecular techniques and information from genetic markers revealed that morphological and historical differences between populations are often mismatched (reviewed in Zink 2004). This is because morphological differences can also be the product of phenotypic plasticity, i.e. the capacity of a genotype to produce different phenotypes in response to environmental variation (Forsman 2015; West-Eberhard 1989) or convergent evolution (Odum 1971) in which case mismatched patterns confuse the understanding of evolutionary history and delineation of conservation units (e.g. Phillimore *et al.* 2008; Walsh *et al.* 2017).

Behavioural traits can also evidence marked differences between populations (Beecher 2016; Di-Poi *et al.* 2014; Kleindorfer *et al.* 2006; Mortega *et al.* 2014; Podos 2010). Culturally learned behavioural traits can be subjected to rapid change following colonisation of new habitats (Di-Poi *et al.* 2014; Green *et al.* 2016; Magurran *et al.* 1992), and reinforce differences between isolated populations, particularly when such traits influence mate-choice (Chamberlain *et al.* 2009; Price 2008). Bird song is an excellent example of a culturally inherited behavioural trait that strongly influences mate choice, at least in some species (Catchpole 1987; Searcy 1992; Slabbekoorn & Smith 2002; Thorpe 1958). Because it is, at least partially, culturally inherited, regional or population differences in song can arise rapidly (Baker *et al.* 2003; Nowicki *et al.* 2001) and have been linked to reproductive isolation in several studies (Irwin *et al.* 2001; Mortega *et al.* 2014; Toews & Irwin 2008). Thus, the study of population differences in bird song can provide insight into completed or ongoing speciation (Price 2008). Using multiple lines of evidence and identifying when and why they concur or not seems thus to be a logical and integrated way to classify species.

1.3 Conservation genetics

Conservation genetics is the use of genetic techniques to assess and reduce the extinction risk of vulnerable populations (Allendorf & Luikart 2007; Frankham *et al.* 2009). Conservation genetics techniques can be useful for resolving taxonomic uncertainties and defining conservation management units (Frankham *et al.* 2009; Moritz 1994b; Palsbøll *et al.* 2007; Ryder 1986; Wallace *et al.* 2010), especially in the case of poorly studied species (Frankham *et al.* 2009). This is especially important when populations of a certain species have become isolated or diverged to the point where they represent unique units of genetic diversity (Moritz 1994b). In an attempt to define and quantify conservation units, ecologists created two now widely used concepts: ‘evolutionarily significant units’ (ESU) and ‘conservation management units’ (MU), both defined by genetic criteria (Moritz 1994b; Ryder 1986). In its most used definition, ESUs should be “reciprocally monophyletic for mitochondrial DNA alleles and show significant divergence of allele frequencies at nuclear loci" (Moritz 1994b). On the other hand, MUs are defined as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles" (Moritz 1994b). In practical terms, the difference between the two concepts is that ESUs represent populations separated by a different evolutionary history, whereas MUs apply to populations currently isolated by a lack of gene flow, regardless of their evolutionary history. Although such definitions are not necessarily equivalent to subspecies (Moritz 1994a), they provide a first quantitative basis for defining conservation priorities.

From a conservation point of view, it is important to understand population viability and potential extinction risks, and conservation genetics is a powerful tool to achieve this (Frankham *et al.* 2009). Small populations generally suffer a higher risk of extinction because they are more vulnerable to effects of stochastic forces, environmental change and natural disasters (Frankham *et al.* 2009). Genetic diversity within a population represents its adaptive potential, i.e. its potential ability to cope with environmental changes (Frankham 2005; Frankham *et al.* 2009). High genetic diversity in a population will help make it more resistant to extinction threats that arise from selective and stochastic pressures (Frankham *et al.* 2009). Maintaining genetic diversity is therefore essential to the conservation of biodiversity.

Genetic factors can also themselves drive population declines and increase extinction risk in the wild (Frankham *et al.* 2009; Saccheri *et al.* 1998; Spielman *et al.* 2004). For example, natural populations with reduced heterozygosity can experience reduced reproductive fitness and elevated extinction risk through loss of any heterozygote advantage (Hostetler *et al.* 2013; Pimm *et al.* 2006; Vilà *et al.* 2003), regardless of initial causes of population decline (Spielman *et al.* 2004). Other problems associated with low genetic diversity and small population size are inbreeding and inbreeding depression (Crnokrak & Roff 1999; Ralls *et al.* 1979; Saccheri *et al.* 1998). Inbreeding is the production of offspring from the mating or breeding of individuals or organisms that are closely related genetically (Allendorf & Luikart 2007; Frankham *et al.* 2009), which can lead to expression of deleterious alleles and loss of heterozygote advantage (Frankham *et al.* 2009; Richardson *et al.* 2004). Inbreeding depression is reduced biological fitness in a given population as a result of inbreeding (Crnokrak & Roff 1999; Ralls *et al.* 1979; Saccheri *et al.* 1998) and has been shown to cause a loss of fitness in various populations of wild organisms (Agudo *et al.* 2012; Briskie & Mackintosh 2004; Saccheri *et al.* 1998).

Genetic markers based on neutral loci, i.e. those not subjected to selective forces, are useful to assess the impact of factors such as genetic drift or gene flow (e.g. Dutta *et al.* 2013; Hille *et al.* 2003; Padilla *et al.* 2015). They are widely used to assess the phylogenetic and phylogeographic history of a species or clade (Avise 2000; Avise 2004; Dool *et al.* 2013; Emerson *et al.* 2001). However, different types of markers have different mutation rates and transmission pathways, revealing different aspects of a species' evolutionary history (Avise 2000; Avise 2004). Microsatellites have been widely used in population and conservation genetics (reviewed in Allendorf & Luikart 2007; Sunnucks 2000). They consist of tandem repeats of a short sequence motif of one to six nucleotides, repeated usually 5 to 100 times which can be analysed by different size migration through gel electrophoresis (Allendorf & Luikart 2007). They are usually highly polymorphic, due to a high mutation rate, typically around 10^{-3} or 10^{-4} per generation (Selkoe & Toonen 2006). Their high mutation rates make them very useful to infer population structure, demography and gene flow over recent evolutionary timescales, i.e. 10 thousand years ago (Kya) to 100 Kya (Dool *et al.* 2013; Ferchaud *et al.* 2015; Illera *et al.* 2007; Xenikoudakis *et al.* 2015) and to assess demographic bottlenecks (Garza & Williamson 2001; Luikart *et al.* 1998; Peery *et al.* 2012; Sunnucks 2000; Williamson-Natesan 2005). In addition to this, the genomic regions flanking microsatellites are generally highly conserved, and primer pairs developed for these regions in one species can often be used in closely related species (Allendorf & Luikart 2007; Hansson & Richardson 2005; Hogan *et al.* 2013; Phillips *et al.* 2013).

In contrast to microsatellites, mitochondrial genes have slower mutation rates, and are commonly used to infer population history over longer time scales, i.e. dating from the Last Glacial Maximum circa 23 Kya to 1 Mya (Avise *et al.* 1987; Clark *et al.* 2009). They can also be used to help determine conservation units, more specifically evolutionarily significant units and management units (Frankham *et al.* 2012; Ryder 1986; Taberlet & Bouvet 1994). Mitochondrial DNA (mtDNA) is especially useful for phylogenetic reconstruction because it is clonal, i.e. maternally inherited, and thus haploid and non-recombining (Allendorf & Luikart 2007; Avise 2004). Hence, it has been frequently used to study population structure and phylogenetic relationships (Galtier *et al.* 2009). It is relatively easy to amplify, its gene content is strongly conserved across animal species, it has no introns and only short intergenic regions and it is highly polymorphic with respect to nuclear loci, because of its elevated mutation rate (Avise 2004; Avise 2009; Galtier *et al.* 2009). Different parts of the mtDNA evolve at different rates, with highly variable regions such as the control region flanked by highly conserved ones (for which PCR primers can be designed) making these suitable markers with which to study genetic variation within and across species (Avise *et al.* 1987; Galtier *et al.* 2009; Moritz *et al.* 1987). However, more recently, the mtDNA's properties of neutrality and clonality, and the clocklike nature of its substitution rate have been questioned (Galtier *et al.* 2009). Additionally, "numts", i.e. nuclear copies of mitochondrial genes, may be present (Bensasson *et al.* 2001; Sorenson & Quinn 1998; Zhang & Hewitt 1996). This a problem for studies aimed at neutral loci, because numts evolve at a different rate and under different constraints compared to real mitochondrial genes (Bensasson *et al.* 2001; Sorenson & Quinn 1998; Zhang & Hewitt 1996). Nevertheless, mitochondrial DNA is one of the most widely used genetic markers in species delimitation, as it evidences reciprocal monophyly much more quickly than nuclear genes (Galtier *et al.* 2009). However, the use of mtDNA alone to delimit species has been criticized, because mtDNA may not show the same patterns as other loci, and because some of the other loci in the same genome may not exhibit reciprocal monophyly when compared to mtDNA (Avise 2004; Edwards *et al.* 2005; Phillimore *et al.* 2008). Because different neutral markers have different properties, combining data from microsatellites and mitochondrial DNA provides a clearer picture of the degree of genetic divergence among populations, and their relative demographic and phylogenetic histories (Reynolds *et al.* 2015).

The relative effects of neutral and adaptive forces in driving population divergence are often difficult to disentangle and quantify (Clegg *et al.* 2002b; Sutton *et al.* 2011; Westerdahl *et al.* 2004). Stochastic forces can shape morphological traits in the absence of or despite deterministic effects of selection (Rocamora & Richardson 2003; Slatkin 1987; Spurgin *et al.* 2014). Indeed, non-adaptive evolution is a significant evolutionary force (Allendorf & Luikart 2007; Hartl & Clark 1997; Kimura & Ota 1974; Wright 1969). Evolutionary differences between isolated populations can be the result of neutral forces such as drift, bottlenecks and founder effects at the genetic level (Kolbe *et al.* 2012; Miller & Lambert 2004; Ramstad *et al.* 2013). Many studies found support for the overriding role of drift in shaping divergence between populations in both genetic and morphological traits (Gonzalez-Quevedo *et al.* 2015; Grueber *et al.* 2013; Miller & Lambert 2004; Spurgin *et al.* 2014). In some cases, a combination of neutral and adaptive forces seems to have shaped divergence of populations and a combination of morphological and genetic research has been crucial in disentangling their impacts under various systems and scenarios (Clegg *et al.* 2002a; Clegg *et al.* 2002b; Clegg & Phillimore 2010; Potvin & Clegg 2015). Mismatches between genetic and phenotypic patterns can indicate action of selective forces, especially when phenotypic patterns match specific environmental factors (Funk *et al.* 2008; Langerhans *et al.* 2003; McKinney *et al.* 2014). In some cases, for example in the Galapagos finches, population divergence has been shown to reflect adaptations to local environments (Grant 1965, 1986; Schluter & Grant 1984). However, it is only possible to identify the action of selective forces with long term studies and/or examination of biologically relevant environmental factors (Bell 2010; Clegg *et al.* 2008; Grant 1986).

1.3 Habitat changes

Assessing divergence among populations in realised ecological niches can be important to understand population extinction risk and conservation priorities (Scheele *et al.* 2017). One dimension of a species' realised niche is their habitat, i.e. the range of physical and biotic parameters for a species' survival (Groom *et al.* 2006; Lindenmayer & Burgman 2005; Primarck 2012). Most commonly, the term habitat is used by ecologists to encompass landscape traits at the locations where a species is present, e.g. altitude, temperature range, precipitation and vegetation composition. Suitable habitat is often the first thing to be studied in a species, because many of its components are easily measurable and can be conserved by direct, straightforward actions. Importantly, habitat changes are one of the biggest and most immediate threats to biodiversity (Butchart *et al.* 2010; Pereira *et al.* 2010). Land use and climate changes are expected to have a large impact on biodiversity loss during the 21st century (Pereira *et al.* 2010). Large scale agricultural activities have been shown to have a negative effect on biodiversity (Donald *et al.* 2001; McLaughlin & Mineau 1995). This might be due to changes in habitat structure or other factors such as introduction of pesticides on the food chain (Mineau & Whiteside 2013). Actions aimed at minimizing land-use change could reduce extinction risks for many species (Pereira *et al.* 2010). However, while management plans can often be applied to protected areas (Dudley 2008), they are more difficult to apply in agricultural areas because of socio-economic conflicts (Giampietro 1997). Recently, policy makers and researchers have begun combining efforts and proposing frameworks to manage agricultural landscapes while protecting biodiversity (Scherr & McNeely 2008; Tanentzap *et al.* 2015).

In addition to agricultural intensification, introduced species i.e. animals, plants and microorganisms, are often a threat for endangered species (Butchart *et al.* 2010; Goodenough 2010). Exotic organisms can compete with native ones, predate on them or introduce diseases (Mooney & Cleland 2001; Sax & Gaines 2008), modify the habitat structure which native species depend on or alter biotic interactions (Simberloff 2011; Totland *et al.* 2006; Trammell & Butler 1995; Wolfe & Klironomos 2005; Zedler & Kercher 2004). For example, Hawaiian honeycreepers are rapidly collapsing due to a multitude of threats (malaria parasites, invasive weeds and non-avian competitors) with effects exacerbated by climate change (Fortini *et al.* 2015; Paxton *et al.* 2016).

1.4 Study system

1.4.1 The Cape Verde islands

The Cape Verde islands are an archipelago of ten islands and several small islets, ca. 500 km off the west coast of Africa (Figure 1.1). These islands have volcanic origin, and their ages are thought to range between ca. 20 Mya, for the eastern islands of Sal, Maio and Boavista, to less than 5 Mya, for the most western islands of Santo Antão, Fogo and Brava (Hazevoet 1995; Pim *et al.* 2008; Ramalho 2011; Torres *et al.* 2002). The archipelago is geographically divided in two main groups of islands: the Barlavento group, on the north, comprising Santo Antão, S. Vicente, S. Nicolau, Sal and Boavista; and the Sotavento, on the south, comprising Brava, Fogo, Santiago and Maio (Correia 1996). The archipelago is located in the Sahel zone (Duarte *et al.* 2008), and is constantly affected by the northeastern trade winds blowing off the west coast of Africa (Hazevoet 1995). The climate is subtropical with two main seasons: the dry season from December to July, and the wet season from August to November (Correia 1996; Hazevoet 1995). Because it is located slightly north of the Intertropical Convergence Zone (Philander *et al.* 1996), the rains brought by the southern monsoon might not always reach the archipelago, or may reach the southern islands but not the northern ones on a given year (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986). Droughts of up to 18 years have been recorded during the last century, and there is anecdotal evidence of desertification over the last centuries (Hazevoet 1995; Sena-Martins & Moreno 1986). During the major drought in the 1940s, up to one quarter of the entire human population of the islands died of famine (Hazevoet 1995; Sena-Martins & Moreno 1986), but there are no records of how the wildlife was impacted.

Ecologically, Cape Verde is part of the Mediterranean Basin biodiversity hotspot (CEPF 2010) and WWF has classified it in a unique ecoregion, the Cape Verde dry forests (Olson *et al.* 2001). Colonisation during the 15th century caused the introduction of many exotic plants and animals. Currently, up to 80% of its flora is non-native, with most native species threatened and confined to small inaccessible areas (Duarte *et al.* 2008; Gomes *et al.* 2003). Agriculture has been constant since colonisation, with the most abundant crops being coffee *Coffea arabica*, sugarcane *Saccharum officinalis* and maize *Zea mays*. The most accepted, but unconfirmed, view is that the archipelago did not support closed canopy woodland before human settlement (Hazevoet 1995). Mountain tops were probably covered in “tortolho” *Euphorbia tuckeyana*, dragon tree *Dracaena drago* and marmulan *Sideroxylon marmulano* (Hazevoet 1995). They were subsequently afforested in the 1930s-40s, and then again between 1975-88 with large numbers of eucalypts *Eucalyptus* spp. and pine trees *Pinus* spp., while the lowlands were planted with mesquite trees *Prosopis juliflora* (Hazevoet 1995). Currently, 21% of the country’s area is agricultural land and 22% planted forest area, compared to only 2.6% of terrestrial area which is under protection (The World Bank 2014a, b, c). There are no permanent freshwater sources in Cape Verde, except for a few streams on Santo Antão and new dams that have been built during the last decade, mostly on Santiago. The main water sources are: 1) the rainy season (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986); 2) the north-eastern trade winds, which bring mist clouds as they encounter the mountains, thus making the north-eastern slopes wetter than the rest of the islands (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986); and 3) artificial irrigation and dams, which are widely used for agriculture (Hazevoet 1995). This unique combination of geographical, geological, climatic and floristic characteristics of the Cape Verde archipelago makes this a rare ecosystem among oceanic archipelagos.

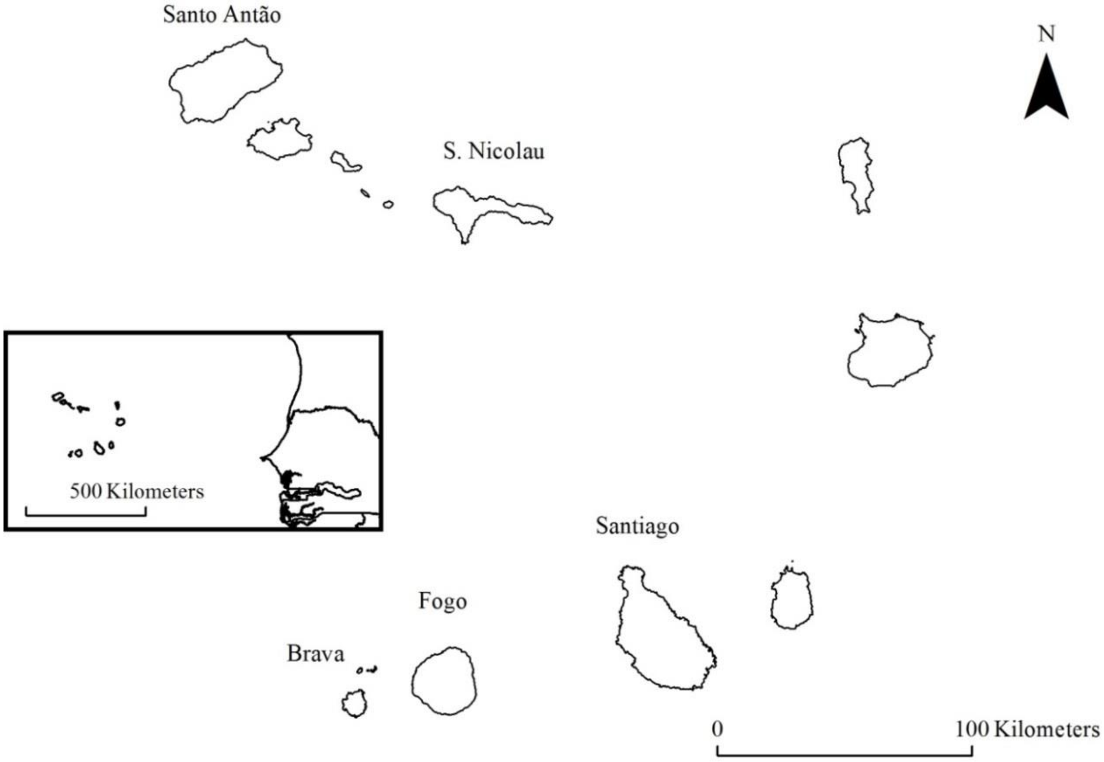

Figure 1.1 Map of the Cape Verde islands (main) with position relative to West Africa (inset). Cape Verde warbler *Acrocephalus brevipennis* populations currently exist on Santiago, Fogo and S. Nicolau, previously existed on Brava until 1969, and were thought to exist on Santo Antão (Hazevoet 1995). *Map produced using open source data on ArcMap 10.1 by Helena Batalha.*

1.4.2 The Cape Verde warbler

The Cape Verde warbler *Acrocephalus brevipennis* is an endangered passerine endemic to Cape Verde (BirdLife International 2016), currently inhabiting Santiago, Fogo and S. Nicolau (Figures 1.1 and 1.2). It is most closely related to the greater swamp warbler *Acrocephalus rufescens* of sub-Saharan Africa (Fregin *et al.* 2009; Leisler *et al.* 1997). The warbler is thought to have inhabited natural scrub vegetation, such as the endemic tortolho *Euphorbia tuckeyana*, before the arrival of European colonisers 500 years ago (Hazevoet 1995). However, it was discovered about 150 years ago on the island of S. Nicolau inhabiting *Arundo donax* reed stands (Keulemans 1866). In the late 19th century the warbler was known to exist on Santiago, S. Nicolau and Brava (Hazevoet 1993). During the 20th century the overall population decreased, possibly due to habitat loss caused by droughts (Hazevoet 1995). By 1995 the Cape Verde warbler was thought to be restricted to a population on Santiago and on the verge of extinction (BirdLife International 2016; Hazevoet 1995). At this time the bird was thought to be restricted to sugarcane *Saccharum officinalis* farmland, coastal plantations and the central vegetated area of S. Jorge dos Órgãos, but absent from the rest of the island (Hazevoet 1995). The warbler was known to still be present on Brava during the early 20th century, where it was found in gardens in the main town but not in irrigated agricultural areas (Bourne 1955; Hazevoet 1995). The last known record from Brava dates from 1969 (Hazevoet 1995). Rumours of the warbler's existence on Santo Antão have never been confirmed (Hazevoet 1995). In 1998 the bird was rediscovered on S. Nicolau (Hazevoet *et al.* 1999), where recent estimates suggested just 8–10 breeding pairs remain (Donald *et al.* 2004; Hazevoet *et al.* 1999). On S. Nicolau, the Cape Verde warbler seems to be restricted to abandoned reed patches in the central area of the island and in valleys with *Mangifera indica* mango trees (Donald *et al.* 2004; Hazevoet *et al.* 1999). In 2004, another warbler population, estimated at the time at ca. 500 breeding pairs, was found on Fogo (Hering & Fuchs 2009; Hering & Hering 2005). This population was found in the extensive coffee plantations interspersed with maize and fruit trees on the northeastern slopes of the island (Hering & Fuchs 2009; Hering & Hering 2005).

Very little is currently known about many aspects of the Cape Verde warbler's biology, including morphology, phenology, behaviour, song or genetics. The only genetic studies undertaken so far have used samples from Santiago birds to determine the Cape Verde warbler's phylogenetic relationships with other members of the *Acrocephalus* genus (Fregin *et al.* 2009; Leisler *et al.* 1997). However the genetic diversity and divergence between these three populations have not yet been assessed. To date, there have been no recorded measurements of live birds (Garcia-del-Rey 2016) apart from a few measurements taken on S. Nicolau when the bird was discovered 150 years ago (Dohrn 1871; Keulemans 1866). Moult patterns are unknown. However, it seems clear that juveniles have a more rufous plumage than greyer adults (Cramp & Perrins 1992). The breeding biology of this species is poorly understood, with few anecdotal observations reporting a typical *Acrocephalus* nest shape where birds lay two to three eggs, but no records of breeding success (Cramp & Perrins 1992). The bird is thought to breed after the first rains, i.e. usually between August and November, but there are some records of breeding attempts in February (Cramp & Perrins 1992; Hazevoet 1995). However, males are thought to be territorial throughout the year (Cramp & Perrins 1992; Hazevoet 1995; Hazevoet *et al.* 1999) and there is anecdotal evidence that the pair bonding season takes place during the dry season, i.e. in April-May (A. Rendall, pers. comm.; Cramp & Perrins 1992). The Cape Verde warbler's song is said to be similar to that of its sister species, the greater swamp warbler (Cramp & Perrins 1992; Hazevoet 1995), but to date no comparative study has been undertaken. Preliminary observations indicated that there is considerable inter- and intra-individual variation in bird song within each island (Cramp & Perrins 1992; pers. obs.).

Figure 1.2 Adult Cape Verde warblers *Acrocephalus brevipennis* on natural habitat. a) Cidade Velha, Santiago, 2013; b) Mosteiros, Fogo, 2016; c) Canto Fajã, S. Nicolau, 2016.

Currently, the Cape Verde warbler's global population is estimated at a maximum of 2,200–3,000 individuals, and is thought to be decreasing (BirdLife International 2016), but lack of census information means that such estimates may be somewhat unreliable (BirdLife International 2016). The species has legal protection status in Cape Verde but to date no countrywide conservation strategy has been developed. There is a clear need for an in-depth and considered species assessment, in order that such a strategy can be formulated and implemented on sound evidence. Herein lies the scope of this thesis, to address this knowledge gap and provide information for the future conservation of the Cape Verde warbler.

In this thesis, I investigate levels of divergence among the three remaining populations of the Cape Verde warbler to understand levels of variation within and among populations, define conservation units and thus inform conservation. To do this I focus on four different and complimentary aspects, i.e. genetics, song, habitat and morphology. In Chapter 2, I assess neutral genetic diversity within each population and divergence among populations using microsatellites and mitochondrial DNA. The data will also allow me to estimate when the three populations became isolated and started to diverge, and whether the populations have undergone recent bottlenecks. In Chapter 3, I characterize warbler song on each of the three islands. I then assess divergence in spectral characteristics of the song to understand this aspect of population divergence. In Chapter 4, I create maps of the predicted suitable area of distribution for the Cape Verde warbler on each island and I use habitat traits to assess if there are differences in habitat use/preference on the three islands and try to understand what key features predict habitat use by the warbler. In Chapter 5, I investigate body size and shape differences between the three populations of the Cape Verde warbler and try to understand if morphological differences match genetic patterns. Finally, in Chapter 6 I discuss my findings from Chapters 2 to 5 in a context of population divergence and conservation prioritisation and suggest possible directions for future research.

Appendix I is a bilingual report I authored for the Cape Verdean government and the Natural Parks of Serra Malagueta, Fogo and Monte Gordo detailing the objectives and results of the first fieldwork season, with the aim of reinforcing good relationships between UK and Cape Verde stakeholders.

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Chapter 2

Genetic diversity and divergence in the endangered Cape Verde warbler

Acrocephalus brevipennis

A version of this manuscript is published in *Conservation Genetics* 18: 343-357.

doi:10.1007/s10592-016-0909-3

Pair of Cape Verde warblers Acrocephalus brevipennis *feeding on sugarcane* Saccharum officinalis*, plus another warbler in the lower left corner, in Cidade Velha, Santiago. Photo taken on November 2013.*

2.1 Abstract

Genetic factors play an important role in the long-term persistence of populations and species, and conservation strategies should take such factors into account. We use neutral molecular markers to assess diversity and divergence between the three remaining island populations of a little-studied endemic passerine, the Cape Verde warbler, *Acrocephalus brevipennis*. Variation at both microsatellite loci and the cytochrome b gene reveal low diversity within the species overall, but considerable divergence among the populations on Santiago, Fogo and São Nicolau islands. The genetic markers show a gradient of genetic diversity with population size, with the smallest population of S. Nicolau being the least diverse, and the largest, Santiago, the most diverse. The more geographically isolated population on S. Nicolau is also more divergent from Santiago and Fogo than these two are from each other. The Cape Verde warbler diverged from its sister species, the greater swamp warbler *Acrocephalus rufescens*, within the last million years, and the three populations became isolated from one another 165,000–199,000 years ago. There is also evidence of population bottlenecks, especially in the smallest and most isolated population of S. Nicolau. This population seems to have decreased during the last century, with potential for further decreases and even extinction. As the three populations are genetically distinct, with no evidence of gene flow between them, we argue they should be treated as separate management units for the successful conservation of this species.

2.2 Introduction

Over the last few centuries many once common species have become threatened or extinct throughout the world, mainly due to anthropogenic impacts (Baillie *et al.* 2004; Butchart *et al.* 2010). Many of these declines and extinctions have occurred on islands (Brooks *et al.* 2002; Sax & Gaines 2008; Steadman 1995). Island species tend to be more vulnerable to extinction threats than mainland species for many reasons (Frankham 1998; Sax & Gaines 2008), including smaller population and range sizes, increased vulnerability to stochastic events, evolutionary/prey naivety, lower levels of genetic diversity and decreased gene flow (Frankham 1998; Frankham *et al.* 2009; Frankham & Ralls 1998; Griffin *et al.* 2000; Sax & Gaines 2008; Sih *et al.* 2010). Islands and archipelagos are often the focus of conservation actions not only because they contain threatened species but also because they can be biodiversity hotspots due to their high levels of endemism (Emerson 2002; Kier *et al.* 2009; Myers *et al.* 2000). Genetic factors can contribute significantly to extinction risk in the wild (Frankham 2005; Frankham & Ralls 1998; Saccheri *et al.* 1998; Spielman *et al.* 2004). Higher levels of genetic variation within a population provide more potential to adapt, for example to environmental changes and novel predators and parasites, whereas small, genetically depauperate populations are disadvantaged in this regard, and can also suffer from inbreeding depression (Brook *et al.* 2002; Crnokrak & Roff 1999; Hedrick & Kalinowski 2000). Such factors can drive small populations into an extinction vortex (Gilpin & Soulé 1986). Consequently, where possible, management plans for threatened species should take genetic factors into account (Brook *et al.* 2002; Frankham 2005).

Genetic data can be used to resolve taxonomic uncertainties, define management units and assess the extinction risk of a species, being therefore important in informing conservation and helping mitigate extinction (Frankham *et al.* 2009). This is especially important when populations have become isolated from one another, or fragmented (Fouquet *et al.* 2010; Garcia-del-Rey *et al.* 2013; Moritz 1994b). In an attempt to develop a framework for prioritizing conservation efforts, the terms 'evolutionarily significant units' (ESUs) and conservation 'management units' (MUs), mostly defined by genetic criteria, were coined (Moritz 1994a; Ryder 1986). Although there are many different definitions (see Fraser & Bernatchez 2001; Funk *et al.* 2012 for reviews), in a genetic framework ESUs should be reciprocally monophyletic for mitochondrial DNA and significantly divergent at nuclear loci (Avise 2004; Moritz 1994b), whereas the concept of MUs is applied to populations which are significantly divergent at nuclear or mitochondrial loci (Moritz 1994a; Ryder 1986). In other terms, ESUs identify historically isolated populations, and MUs can be defined as populations which show incomplete phylogenetic divergence but currently lack gene flow (Moritz 1994b). Although such definitions are not necessarily equivalent to subspecies (Moritz 1994b), they provide a first quantitative basis for defining conservation priorities below the species level (Funk *et al.* 2012).

Different types of genetic markers can have different mutation and recombination rates or transmission pathways, thus they can reveal different aspects of a species' evolutionary history (Avise 2004). Microsatellites have fast mutation rates (Selkoe & Toonen 2006), and can be used to infer current population structure, demography and gene flow (e.g., Dool *et al.* 2013; Ferchaud *et al.* 2015; Illera *et al.* 2007; Xenikoudakis *et al.* 2015). Furthermore, because of their high levels of polymorphism they are also useful for assessing demographic bottlenecks (Garza & Williamson 2001; Sunnucks 2000; Williamson-Natesan 2005). In contrast, mitochondrial DNA has slower mutation rates, and is commonly used to infer population history dating to, and before, the Last Glacial Maximum (Avise *et al.* 1987) circa 23 Kya ago (Clark *et al.* 2009). Combining data from microsatellites and mitochondrial DNA can provide a clearer picture of diversity within, and divergence among, populations, and can help reconstruct their relative demographic and phylogenetic histories (Reynolds *et al.* 2015). Additionally, patterns of variation at neutral markers can provide information about colonisation, gene flow, phylogeny and historical relations between populations (e.g., Dool *et al.* 2013; Dutta *et al.* 2013; Ferchaud *et al.* 2015; Hille *et al.* 2003; Padilla *et al.* 2015). Finally, genetic markers can be used to assess whether a lack of diversity may be contributing to current declines in the population (Allendorf & Luikart 2007; Frankham *et al.* 2009) and to determine if augmentation (Weeks *et al.* 2011) may be required to alleviate current problems, or ensure maximal adaptive potential is retained in the species for the future.

The Cape Verde warbler *Acrocephalus brevipennis* is an endangered passerine endemic to Cape Verde, a volcanic archipelago ca. 500 km off West Africa, between 14–18°N and 22–26°W in the Atlantic Ocean (Figure 2.1). It is most closely related to the greater swamp warbler *Acrocephalus rufescens* of sub-Saharan Africa (Fregin *et al.* 2009; Leisler *et al.* 1997). The Cape Verde warbler was discovered about 150 years ago on the island of São Nicolau (Keulemans 1866), and in the late 19th century it was known to exist at least on Santiago, S. Nicolau and Brava (Hazevoet 1993). During the 20th century the population decreased, possibly due to habitat loss caused by droughts (Hazevoet 1995) and by 1995 the bird was thought to be confined to Santiago (BirdLife International 2016; Hazevoet 1995). However, in 1998 the bird was rediscovered on S. Nicolau (Hazevoet *et al.* 1999), where the most recent published estimates suggested just 8–10 breeding pairs remain (Donald *et al.* 2004; Hazevoet *et al.* 1999). In 2004, another population, estimated at ca. 500 breeding pairs, was found on Fogo (Hering & Fuchs 2009; Hering & Hering 2005). Fogo and Santiago, in the southern Sotavento group of islands, are separated by ca. 60 km, whereas S. Nicolau, in the northwestern Barlavento group, is separated from the former two islands by more than 160 km (Figure 2.1). Such oceanic barriers can prevent gene flow in *Acrocephalus* warblers (Cibois *et al.* 2008; Komdeur *et al.* 2004), especially between the Sotavento and Barlavento groups (Hazevoet 1995; Hille *et al.* 2003), which could have important implications for this species' conservation. Currently, the Cape Verde warbler's global population is estimated at a maximum of 2,200–3,000 individuals, and is thought to be decreasing (BirdLife International 2016), but lack of census information means that such estimates may be somewhat unreliable (BirdLife International 2016). The species has legal protection status in Cape Verde but to date no countrywide conservation strategy has been developed. An understanding of the genetic variation within, and divergence between, the three warbler populations is clearly important to the success of any future conservation programme (Frankham *et al.* 2009; McCartney-Melstad & Shaffer 2015).

Here I use neutral molecular markers (mtDNA and microsatellites) to study the three known remaining populations of the Cape Verde warbler. The aims were to clarify the degree of genetic divergence between the bird and its sister species, the greater swamp warbler, to assess genetic relationships between Cape Verde warbler populations, and to measure genetic diversity within each population so that this knowledge can be used to inform future conservation actions.

2.3 Materials and methods

2.3.1 Sample collection and DNA extraction

Samples were collected from November 2013 to January 2014, and September to November 2014, the main breeding season (Hazevoet 1995). I surveyed for warblers across all potential suitable habitat types and altitudes (the maximum altitude where a bird was sampled was 1,384 m above sea level) on each of the three islands to ensure that coverage was as comprehensive as possible (Figure 2.1, Table S2.1). We also conducted extensive transect and point counts in January 2014 on Brava (2 people x 4 days), where the bird was present until 1969 (Hazevoet 1995), and in September 2014 on Santo Antão (2 people x 7 days), where the vegetated valleys were thought to provide suitable habitat (Hazevoet 1995). When detected, birds were attracted with conspecific song playback and caught with mist nets. Each caught bird was fitted with a unique combination of a numbered Cape Verde metal ring, and three UV-resistant plastic colour rings. We determined if the birds were adults or juveniles based on previously described criteria for this and other warbler species (Baker 1997; Cramp & Perrins 1992; Komdeur 1991), and recorded each sampling location with a Garmin eTrex® H GPS. A small ca. 40 µl blood sample was collected from each bird by brachial venipuncture and stored at room temperature in 800 µl absolute ethanol. We aimed to sample a minimum of 25–30 unrelated individuals per island, as this is sufficient for accurate estimation of genetic parameters in microsatellite analyses (Hale *et al.* 2012). In addition, we used seven other Cape Verde warbler samples from S. Jorge dos Órgãos (central Santiago), seven greater swamp warbler samples (one from Senegal and six from Nigeria) and three Seychelles warbler *Acrocephalus sechellensis* samples (details on additional samples, sampling sites, dates, and bird age determination in Supplementary Material and Table S2.1). DNA was extracted using a salt extraction protocol following Richardson *et al.* (2001). Individuals were molecularly sexed following Griffiths *et al.* (1998).

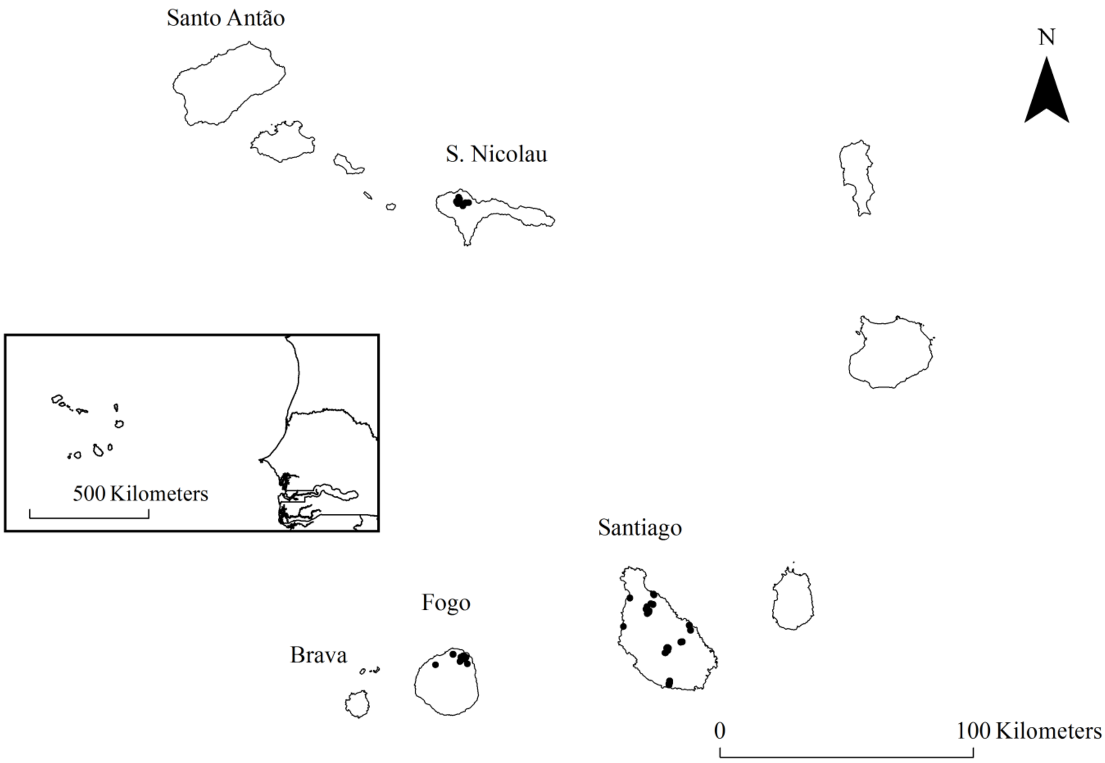

Figure 2.1 The Cape Verde archipelago with the locations where Cape Verde warblers were sampled in 2013 and 2014. Inset shows the archipelago in relation to West Africa. For details on sampling sites and dates, see main text and supplementary material.

2.3.2 Molecular markers

Cytochrome b

We amplified and sequenced a 1,150 bp fragment of mtDNA comprising the last 13 nucleotides of the ND5 gene, 1,128 nucleotides of the cytochrome b, and 9 nucleotides in between the two genes (hereafter “cytochrome b”, for simplicity). To avoid amplifying nuclear copies of the mitochondrial DNA (numts; Sorenson & Quinn 1998), we re-designed the primers ND5 and mt-F from Helbig *et al.* (2005). We used BLAST (Altschul *et al.* 1997) to map the primer sequence against the mitochondrial genome of the Eurasian reed warbler *Acrocephalus scirpaceus*, (accession number AM889139, Singh *et al.* 2008) in GenBank (Benson *et al.* 2013) and re-designed them to match that genome. We checked the redesigned primer pair (ND5-Acro 5'-GGCCTAATCAAAGCCTAC-3' and mt-F-Acro 5'-GGCTTACAAGACCAATGTTT-3') for compatible annealing temperatures, hairpins, complementarity, primer-dimer formation and % GC content using Primer3 (Koressaar & Remm 2007; Untergasser *et al.* 2012). Polymerase chain reactions (PCRs) were performed in 15 µl total volume, consisting of 7.5 µl TopTaq Master Mix (Qiagen, West Sussex, UK), 0.6 µl (10 µM) each primer, 4.8 µl ddH2O and 1.5 µl

DNA. Thermal cycle conditions were 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 1 min, with a final extension step at 72°C for 10 min and a cooling step at 20°C for 5 min. PCR products were quantified in a 1.5% agarose gel and inspected for signs of non-specific annealing, i.e. ghost bands (Bensasson *et al.* 2001; Sorenson & Quinn 1998). Successfully amplified products were cleaned with 5 µl of Exo and FastAP (Life Technologies, UK) to 10 µl of PCR product, and sequenced in both directions (Eurofins, Germany). PCRs were repeated twice to confirm unique haplotypes and to resolve any ambiguous sites.

Microsatellites

Individuals were genotyped at 17 polymorphic microsatellite loci, using multiplex combinations adapted from Spurgin et al. (2014; Table S2), using primers designed for the Seychelles warbler (Richardson *et al.* 2000). Each PCR included 1 µl Qiagen PCR multiplex master mix, 1 µl primer mix and 1 µl pre-dried DNA. The PCR cycling conditions were 15 min at 95°C, followed by 30 cycles of 30 s at 94°C, 1 min 30 s at 55 or 56°C and 1 min at 72°C, followed by a final extension step of 30 min at 60°C. PCR products were separated on an ABI 3730 DNA analyser (Applied Biosystems, UK), and allele sizes were assigned using GeneMapper 4.0 software (Applied Biosystems, UK). Genotypes were checked visually. PCRs were repeated twice to confirm unique alleles. In cases where two or more amplifications resulted in conflicting genotypes for the same individual we considered the individual as heterozygous. We calculated microsatellite error rate per locus according to Pompanon et al. (2005). One of the markers, Ase6, could not be reliably scored due to stutter, so it was excluded from further analyses.

2.3.3 Data analyses

Cytochrome b

Unless stated otherwise, statistical analyses were performed in R v. 2.14.1 (R Core Team 2016). Sequences were visually inspected in FinchTV v. 1.4.0 (Geospiza Inc., Seattle, WA, USA) and manually aligned in BioEdit v. 7.0.9.0 (Hall 1999), against partial CDS sequences of a Cape Verde and a greater swamp warbler (from Senegal) previously published (Fregin *et al.* 2009) – accession numbers FJ883026 and FJ883037, respectively. The sequences were translated in MEGA v. 6 (Tamura *et al.* 2013), and checked for stop codons. We calculated the number of haplotypes and segregating sites, haplotype and nucleotide diversity, Tajima's D (Tajima 1989) and Fu's FS (Fu 1997) in DnaSP v. 5 (Librado & Rozas 2009), and pairwise F_{ST} values (Weir & Cockerham 1984; Wright 1951) in Arlequin v. 3.5 (Excoffier & Lischer 2010). Significance of F_{ST}

values was tested with 10,000 permutations. We calculated mean genetic distances within and among the three populations of Cape Verde warbler and the greater swamp warbler in DnaSP, using the pairwise distance model. A phylogenetic tree was constructed using the maximum likelihood method implemented in MEGA v. 6, based on the Tamura-Nei model of evolution (Tamura & Nei 1993) as selected by the Bayesian Information Criterion in the same software. We used 1,000 bootstrap replicates as a test of phylogeny, and a discrete Gamma distribution with four categories to model evolutionary rate differences among sites. However, haplotype networks are usually more appropriate to visualise relationships between haplotypes in intraspecific datasets than phylogenetic trees (Bandelt *et al.* 1999; Posada & Crandall 2001). Therefore, a median joining haplotype network was also built in Network v. 4.6.1.3 (Bandelt *et al.* 1999).

Time to most recent common ancestor

Time to most recent common ancestor (tMRCA) of the Cape Verde and greater swamp warblers was estimated using a Bayesian phylogenetic approach in BEAST v.2.3.0 (Bouckaert *et al.* 2014; Drummond *et al.* 2012). We estimated the best-fit model of nucleotide substitution in jModelTest v. 2.17 (Darriba *et al.* 2012; Guindon & Gascuel 2003), which tests for the best model of evolution using the Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC) and decision theory (DT). AIC selected GTR+I whereas BIC and DT selected HKY+G. We therefore used the HKY model of substitution (Hasegawa *et al.* 1985) with a gamma site model comprising four rate categories (Yang 1994) and an estimated gamma shape parameter. Effective sample sizes (ESS) for all model parameters exceeded the recommended minimum 200 when using the HKY model, so this model was considered appropriate. The dataset was composed of unique haplotypes of Cape Verde and greater swamp warblers, using the Seychelles warbler as an outgroup. Codons were partitioned into 3 (1 + 2 + 3, frame 2) to allow each nucleotide position within the codon to be modelled separately. Empirical substitution model state frequencies were used along with an estimated HKY kappa parameter. We used a substitution rate of 0.0105 per site per million years, which corresponds to a 2.1% divergence rate, generally applicable to passerine cytochrome b sequences (Weir & Schluter 2008). Molecular clock rate variation is highly unlikely in our dataset, which comprises a single gene across closely related species with resulting low sequence information content. We therefore employed a strict molecular clock. As neither fossil nor relevant biogeographical calibration points were available for this clade of passerines, we utilised published molecular phylogenies to provide loose bounds for the priors. We specified a uniform distribution between 1 and 12

Mya for the tMRCA of the Cape Verde, greater swamp and Seychelles warblers group, as 12 Mya is the estimated divergence time of the small and large groups of *Acrocephalus* warblers; Cape Verde, greater swamp and Seychelles warblers are members of the latter group (Leisler & Schulze-Hagen 2011; Price 2008). We used a diffuse uniform prior bound between the present and 1 Mya to estimate tMRCA of Cape Verde and greater swamp warblers and the same for the tMRCA of the three Cape Verde warbler populations. This is because, even though a divergence time has never been proposed, previous phylogenetic analyses suggests that these two sister species are so similar, when compared to the dated splits in the *Acrocephalus* phylogeny, that they are not likely to have diverged more than 1 Mya (Fregin *et al.* 2009; Leisler *et al.* 1997; Leisler & Schulze-Hagen 2011). Finally, the birth rate and clock rate priors were both assigned diffuse gamma distributions ($\alpha = 0.001$, $\beta = 1000$) (Drummond & Bouckaert 2014). The Markov chain Monte Carlo (MCMC) simulations were run with a standard 10% burn-in. We did four runs, with a total of 100 million iterations, to check mixing and convergence, which was assessed with ESS. Runs were combined using LogCombiner (Rambaut & Drummond 2015) and the results were visualised using Tracer v.1.6 (Rambaut *et al.* 2015).

Microsatellite analysis

For each microsatellite locus and population we tested for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP v. 4.0.10 (Raymond & Rousset 1995). We applied a Bonferroni sequential correction to control for type I errors (Rice 1989). Null allele estimates were calculated in MICROCHECKER (van Oosterhout *et al.* 2004). Allelic richness and number of private alleles in each population were calculated after controlling for differences in sample size, using a rarefaction approach implemented in HP-RARE (Kalinowski 2005). Inbreeding coefficients (F_{IS}) were calculated with GENEPOP. We tested for population structure by calculating global and pairwise F_{ST} values across the three Cape Verde warbler populations. Global and pairwise F_{ST} estimates, observed (Ho) and expected (He) heterozygosity were calculated using Arlequin v. 3.5 (Excoffier & Lischer 2010).

To determine the most likely number of genetic clusters (K), a Bayesian algorithm was implemented in STRUCTURE v. 2.3 (Pritchard *et al.* 2000). The goal was to detect overall population structure in the Cape Verde warbler, so we included information on population of origin in the analysis. We used a no-admixture ancestry model and independent allele frequencies. However, as a no-prior model may be better for revealing subtle similarities, or possible gene flow between populations (Falush *et al.* 2007; Hubisz *et al.* 2009; Pritchard *et al.* 2000), we also ran a model using admixture and correlated allele frequencies, disregarding prior information on sampling location. For both models, we undertook four runs of 500,000 MCMC repetitions with a burn-in of 20,000 at each clustering level for K = 1–6. To assess structure within the Santiago population, we ran both analyses using only the Santiago birds. The number of clusters best fitting the data was determined using both log probabilities and the ad hoc ΔK test (Evanno *et al.* 2005), implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012). Graphical results were visualised using DISTRUCT version 1.1 (Rosenberg 2004).

Bottleneck tests

We used three methods to assess evidence of population bottlenecks: the heterozygosity excess test (Cornuet & Luikart 1996) implemented in BOTTLENECK v. 1.2.02 (Piry *et al.* 1999); the mode shift test (Luikart *et al.* 1998), implemented in the same software; and the M-ratio test (Garza & Williamson 2001), as well as a modified version of this last test which accounts for monomorphic alleles (Excoffier *et al.* 2005), in Arlequin. Heterozygosity and mode-shift tests use excess heterozygosity and allele frequency data to detect recent bottlenecks, i.e. bottlenecks that have occurred within the past dozen generations (Luikart *et al.* 1998). M-ratio tests (Excoffier *et al.* 2005; Garza & Williamson 2001) are based on the reasoning that a bottlenecked population will lose some rare alleles, and this loss will not depend on allele size; therefore a bottlenecked population will show gaps in the allele size distribution. M-ratio tests can detect bottleneck signatures over longer periods than heterozygosity or shift mode tests, as M-ratios are likely to change more slowly than heterozygosity after a bottleneck (Garza & Williamson 2001; Peery *et al.* 2012; Williamson-Natesan 2005).

The distances between these islands are unequal (see Figure 2.1), and dispersal patterns can be differently influenced by the direction of the prevailing winds (Correia 1996; Hazevoet 1995; Hille *et al.* 2003). The islands have different geography, age, and habitat types available for the birds (Hazevoet 1995) and there are only three populations. It is therefore not possible to ascertain the effects of isolation by distance (Wright 1943), resistance (a metric which combines Euclidean distances and spatial heterogeneity) (McRae & Nürnberger 2006) or environment factors (Wang & Bradburd 2014) on the genetic structure of the Cape Verde warbler using these data.

2.4 Results

Cytochrome b

We observed 16 cytochrome b haplotypes in the Cape Verde warbler, five haplotypes in the greater swamp warbler and one haplotype in the Seychelles warbler (Table 2.1; Figures 2.2 and 2.3). The Cape Verde warbler populations do not share haplotypes except for the most common one, shared between Santiago and Fogo (Figures 2.2 and 2.3). The sequences have been deposited in the National Center for Biotechnology Information (NCBI) GenBank database (accession nos. KX518324–KX518347). Where the same haplotype was found in two different populations, we submitted two identical sequences. Basic diversity indexes are given in Table 2.1. Pairwise genetic distances between haplotype sequences within and among Cape Verde warbler populations were very low in all cases, ranging between 0.1% and 0.5% (Table 2.2). The Santiago population (the largest) showed the highest diversity within the Cape Verde warbler, in terms of number of haplotypes, number of segregating sites, haplotype and nucleotide diversity (Table 2.1, Figure 2.4). Nevertheless, haplotype and nucleotide diversity in the greater swamp warbler were much higher, despite the limited number of samples screened (Table 2.1). The population of S. Nicolau exhibits the lowest levels of diversity, while Fogo is intermediate (Table 2.1, Figure 2.4). Diversity levels for the Cape Verde warbler population overall are similar to those for the Santiago population (Table 2.1). The neutrality tests (Tajima's D and Fu’s FS), which reflect population expansion or contraction in the absence of selective forces, were not significant but showed tendencies for population expansion on Santiago, and contraction on Fogo and S. Nicolau (Table 2.1).

Table 2.1 Genetic diversity and demography in the Cape Verde warbler (CVW) and greater swamp warbler (GSW) based on cytochrome b; N = number of individuals sequenced; S = segregating sites; h = number of haplotypes; Hd = haplotype diversity; Pi = nucleotide diversity; for Tajima’s D, all *P* > 0.05 and for Fu’s FS, all *P* > 0.02 (non-significant).

Population	N	S	h	Hd ± SD	Pi ± SD	Tajima's D	Fu's FS
All CVW	145	17	16	0.887 ± 0.011	0.00182 ± 0.00009	-0.887	-4.335
Santiago	72	13	12	0.841 ± 0.024	0.00134 ± 0.00011	-1.222	-4.356
Fogo	43	4	3	0.642 ± 0.035	0.00172 ± 0.00008	2.601	4.450
S. Nicolau	30	3	2	0.370 ± 0.084	0.00097 ± 0.00022	1.097	3.704
GSW	7	8	5	0.905 ± 0.103	0.00315 ± 0.00063	0.569	-0.333

Table 2.2 Estimates of evolutionary divergence among haplotype pairs (pairwise genetic distances), between the Cape Verde warbler populations and greater swamp warbler (GSW), based on cytochrome b sequences: within (diagonal in underlined italic) and among population (below diagonal).

Population	**Santiago**	**Fogo**	**S. Nicolau**	**GSW**
Santiago	*0.001*			
Fogo	0.002	*0.002*		
S. Nicolau	0.002	0.002	*0.001*	
GSW	0.004	0.005	0.005	*0.003*

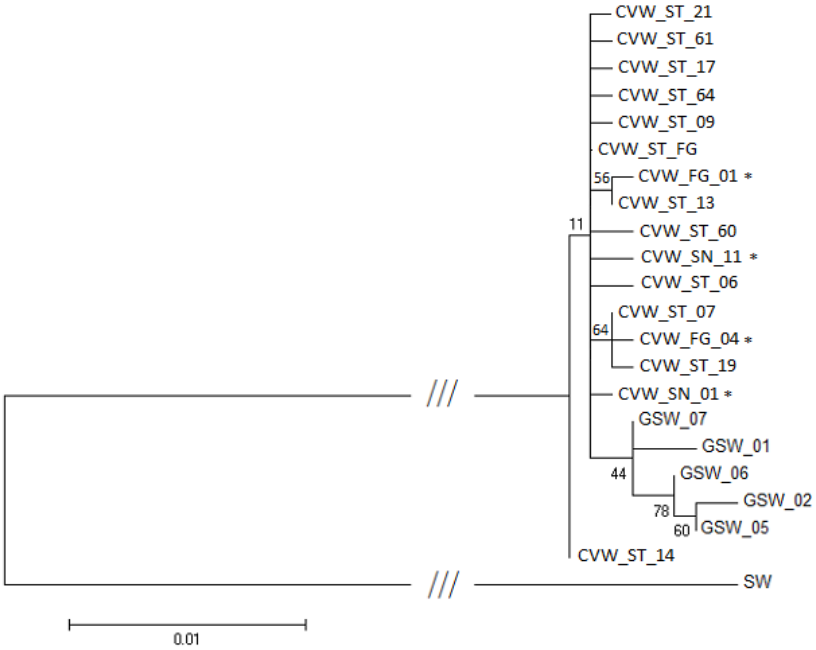

Figure 2.2 Maximum likelihood phylogenetic analysis of the Cape Verde warbler (CVW, n = 145) and greater swamp warbler (GSW, n = 7) species, based on cytochrome b; haplotypes names are coded by island (ST = Santiago, FG = Fogo, SN = S. Nicolau); asterisks mark haplotypes only found on Fogo or S. Nicolau; the Seychelles warbler haplotype (SW, n = 3) is used as an outgroup.

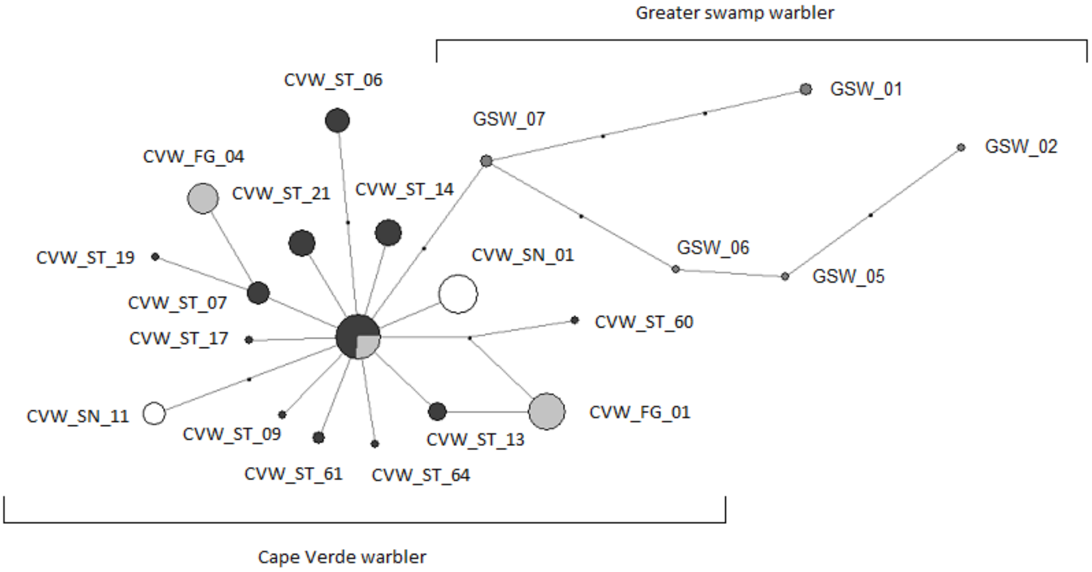

Figure 2.3 Median-joining haplotype network based on cytochrome b of the Cape Verde warbler (CVW, n = 145) and the greater swamp warbler (GSW, n = 7). Haplotypes found in the Cape Verde warbler populations are represented in dark grey for Santiago, light grey for Fogo and white for S. Nicolau; the central haplotype is the most common one (labelled CVW_ST_FG in Figure 2.2); haplotypes found in the greater swamp warbler, in medium grey, cluster separately. The size of the circles is proportional to the number of individuals that share that haplotype. Black dots represent intermediate (unsampled) haplotypes, with 1 bp between consecutive haplotypes.

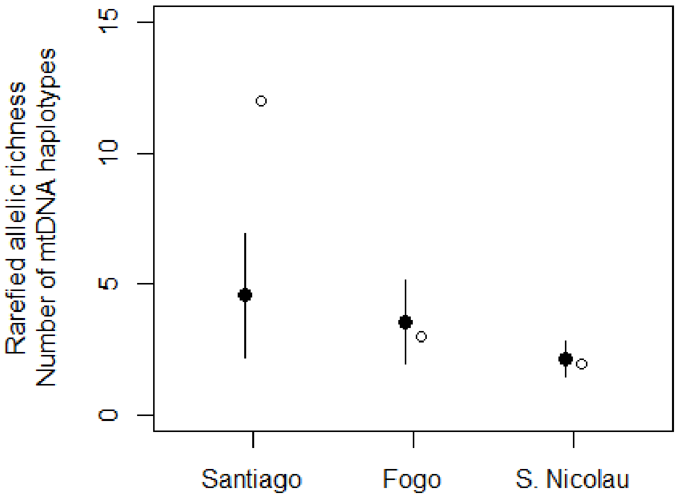

Figure 2.4 Gradient of decreasing genetic diversity with estimated population size: rarefied allelic richness (full circles, n = 131; error bars = SD) and number of haplotypes (empty circles, n = 145) for each of the Cape Verde warbler (CVW) populations, ordered by decreasing population size from Santiago to S. Nicolau.

The maximum likelihood phylogenetic tree and the haplotype network show that the greater swamp warbler haplotypes cluster in one distinct group (Figures 2.2 and 2.3). Among the Cape Verde warbler haplotypes, some nodes have low bootstrap support values because of the low information content of the sequences, as seen in their very low genetic distances (Table 2.2). Importantly, the S. Nicolau haplotypes seem to have derived from the most common haplotype (labelled CVW_ST_FG in Figure 2.2), which is found on Santiago and Fogo but not on S. Nicolau (Figures 2.2 and 2.3). Apart from this most common haplotype, there are no shared haplotypes between the three populations.

Time to the most recent common ancestor

The tMRCA estimate was sampled from an approximately log-normal distribution; hence geometric means are provided (Morrison 2008). The tMRCA for the Cape Verde and greater swamp warbler was estimated at about 292 Kya, with upper confidence limits well within the last million years (Table 2.3). The tMRCA of S. Nicolau and the two Sotavento populations was estimated at 199 Kya, and the tMRCA for Santiago and Fogo at 165 Kya, both with upper 95% confidence limits within the last 650 thousand years (Table 2.3). The lower 95% confidence limits for the tMRCA for all Cape Verde warbler populations, and for the Sotavento populations, was over 30 Kya ago, i.e., prior to the Last Glacial Maximum (Clark *et al.* 2009).

Table 2.3 Estimates of the time to the most recent common ancestor (tMRCA; geometric mean shown) for the Seychelles warbler (SW), Cape Verde warbler (CVW) and greater swamp warbler (GSW), based on the cytochrome b; HPD = highest posterior density; Mya = million years; Kya = thousand years.

Split	**tMRCA**	**95% HPD**
Seychelles warbler / Cape Verde warbler and greater swamp warbler	3.23 Mya	1.00–9.63 Mya
Cape Verde warbler / greater swamp warbler	292 Kya	67–872 Kya
S. Nicolau / Santiago and Fogo	199 Kya	40–615 Kya
Santiago / Fogo	165 Kya	32–511 Kya

Microsatellites

Genetic diversity measurements are given in Table 2.4. The estimated error rate per locus was 0.25%. Evidence for null alleles was found in microsatellite loci Ase11 and Ase22 with null allele frequencies of 0.309 and 0.164, respectively, using the Oosterhout algorithm (van Oosterhout *et al.* 2004), so these were removed from subsequent analyses. Ase13 and Ase48 were in strong linkage disequilibrium (P < 0.001) across all populations. There was no difference between results of summary statistics using either only Ase13 or only Ase48 (Mantel test on F_{ST} matrices, P = 0.172; Wilcoxon tests on He and Ho, all P > 0.742), so Ase48 was removed from further analyses. After removing these markers, we found no deviations from HWE or significant LD between loci. Heterozygosity, allelic richness and number of private alleles show a clear gradient, with highest values in Santiago, intermediate in Fogo and lowest in S. Nicolau, concurring with the mtDNA results (Table 2.4 and Figure 2.4). The inbreeding coefficient (F_{is}) is very close to random expectation for all the populations of the Cape Verde warbler, i.e. between -0.0030 and 0.0047 (Table 2.4).

Table 2.4 Genetic diversity for the Cape Verde warbler (CVW) populations, based on 13 microsatellite loci and using only unrelated birds (N); observed (Ho) and expected (He) heterozygosity, allelic richness, private alleles and inbreeding coefficient (F_{IS}).

Population	N	Ho ± SD	He ± SD	Allelic richness ± SD	Private alleles ± SD	F_{IS}
All CVW	131	0.483 ±0.181	0.543 ±0.207	5.536 ±3327	na	0.1100
Santiago	66	0.567 ±0.196	0.565 ±0.195	4.598 ±2.446	1.290 ±1.271	-0.0030
Fogo	38	0.507 ±0.241	0.509 ±0.228	3.574 ±1.651	0.362 ±0.596	0.0047
S. Nicolau	27	0.355 ±0.120	0.355 ±0.126	2.151 ±0.685	0.183 ±0.376	0.0018

Global F_{ST} was 0.33 for cytochrome b and 0.16 for microsatellites (both P < 0.001), showing pronounced overall differentiation among the populations. Pairwise F_{ST} values are shown in Table 2.5. Differentiation between the Santiago and Fogo populations was moderate to high, and high between those populations and that of S. Nicolau. The pattern of differentiation identified among populations was the same for mitochondrial and microsatellite markers.

Table 2.5 Pairwise F_{ST} values for the Cape Verde warbler populations of Santiago, Fogo and S. Nicolau showing microsatellite pairwise F_{ST} above the solid line and Cytochrome b F_{ST} values below; all values are significant at P < 0.001.

Population	Santiago	Fogo	S. Nicolau
Santiago		0.062	0.232
Fogo	0.256		0.275
S. Nicolau	0.349	0.450	

The Evanno method supports two main genetic clusters (k = 2; highest ΔK = 342.51, Figures S2.1 and S2.2), one including the S. Nicolau population and one with the Sotavento populations (Santiago and Fogo, Figure S2.2). However, when k = 3 the plot shows clear separation between the three island populations (Figure 2.5). This indicates that even though the main differences are between S. Nicolau and the Sotavento populations, there are also marked differences between Santiago and Fogo. For the analyses using only the Santiago population, the best k = 1 (results not shown), showing no evidence of structure within the Santiago population.

Figure 2.5 STRUCTURE plot showing k = 3 distinct genetic clusters in the Cape Verde warbler samples where Santiago, n = 66; Fogo, n = 38; and S. Nicolau, n = 27. Each bar represents an individual's proportional probability of membership to each cluster, given in dark grey, light grey and white, respectively.

Bottleneck tests

All tests show a clear signal of a past population bottleneck in the S. Nicolau population (Table 2.6). The M-ratio tests and modified M-ratio tests range between 0.15 and 0.30 (Table 2.6), well below the critical value of 0.68 (Garza & Williamson 2001), suggesting bottlenecks have occurred on all islands. However the shift mode and heterozygosity tests (using the strict stepwise mutation model) did not detect bottlenecks for the Santiago and Fogo populations (Table 2.6).

Table 2.6 Wilcoxon test for heterozygosity excess *P*-values (IAM = infinite allele model; TPM = two phase model with either 70% or 90% of stepwise mutations and SMM = stepwise mutation model), mode shift test and Garza-Williamson's M and modified M ratio indexes for bottleneck detection in the three Cape Verde warbler populations.

		TPM			Shift		
Population	**IAM**	**(70%)**	**(90%)**	**SMM**	**mode test**	**M index**	**Modified M index**
Santiago	0.0006	0.0210	0.0327	0.1465	no	0.307 ±0.117	0.289 ±0.120
Fogo	0.0002	0.0057	0.0061	0.0640	no	0.272 ±0.135	0.221 ±0.098
S. Nicolau	0.0002	0.0005	0.0005	0.0024	yes	0.201 ±0.106	0.151 ±0.101

2.5 Discussion

Overall we found low genetic diversity within, but considerable divergence among, the three remaining populations of Cape Verde warbler. There is a gradient of genetic diversity decreasing from Santiago through Fogo to S. Nicolau (Figure 2.4). The gradient of genetic diversity from the larger to the smaller island is consistent with expectations derived from population and island size theories (Frankham 1996; Frankham *et al.* 2009; MacArthur & Wilson 1967). Santiago possibly holds the largest population, estimated at ca. 500 pairs in the 1980s, when the warbler was thought to be absent from the north part of the island (Hazevoet 1995). As birds can now be found all across this island, if the density and territory size are relatively constant, the actual population size is likely to be considerably higher (pers. obs.). However, to our knowledge there is no information on density or territory sizes for this island, and no censuses that could provide a reliable basis for an accurate estimate have been carried out on Santiago; thus estimates must be taken with extreme caution (BirdLife International 2016). Clearly, it would be advisable to undertake thorough population censuses on Santiago to better inform conservation. The population on Fogo is medium-sized, with ca. 500 pairs (Hering & Fuchs 2009) and birds limited to the well-vegetated north-eastern part of the island. On S. Nicolau there is a very small population (8-10 pairs; Donald *et al.* 2004; Hazevoet *et al.* 1999, 12-13 pairs; this study) limited to the reed patches and mango tree valleys within a small (< 20 km^2) central part of the island (pers. obs.).

Genetic divergence is greatest between S. Nicolau and the two Sotavento populations of Santiago and Fogo (Table 2.5). Our results are similar to those of previous studies addressing genetic divergence in other Cape Verdean taxa including geckos (Arnold *et al.* 2008; Vasconcelos *et al.* 2012), skinks (Brehm *et al.* 2001; Brown *et al.* 2001) and kestrels (Hille *et al.* 2003). There is usually a clear genetic differentiation between the populations on the Barlavento and Sotavento island groups (e.g., Brown *et al.* 2001; Hille *et al.* 2003), except where the focal species occupy micro-habitat niches and/or have very limited dispersal capacities. In such cases, they can be genetically divergent even among closely located islands (Arnold *et al.* 2008; Brehm *et al.* 2001; Vasconcelos *et al.* 2012); nevertheless the differentiation between Barlavento and Sotavento island groups is always present.

The warbler population on Santiago is widespread, inhabits a diverse range of habitats, from sugarcane plantations to mountain forests, and is genetically the most diverse of the three

(Tables 2.1 and 2.5, Figures 2.3 and 2.4), but we did not find any population substructure. Considering that, based on area of occurrence of the warbler alone, it is potentially the largest population, and the fact that it is the most genetically diverse, we would argue that the Santiago population is currently of least conservation concern of the three populations. Although the Fogo population was only discovered in 2004 (Hering & Hering 2005), our results indicate that the island was colonised well before the Last Glacial Maximum (> 30 Kya) and has been isolated and diverging ever since. This population seems to be of medium conservation concern. Taken together, our results indicate that the split between the Santiago and Fogo populations was more recent than between those two and S. Nicolau. Current demographic trends in any of the three populations are unknown.

When the Cape Verde warbler was discovered on S. Nicolau in the 1860s, it was said to be “numerous” (Keulemans 1866). However, by the 1920s it was considered extinct on the island, and was only rediscovered in the 1990s (Hazevoet 1995; Hazevoet *et al.* 1999). One specimen, collected in the 1970s and housed in the Zoological Centre in Lisbon, is testimony that a remnant population persisted between the 1920s and the 1990s (Hazevoet *et al.* 1999). Our results support the occurrence of a severe bottleneck in this population, in agreement with the anecdotal evidence (Donald *et al.* 2004; Hazevoet *et al.* 1999). This population has lower diversity, different microsatellite allele frequencies and private mitochondrial alleles when compared to Santiago and Fogo. Phylogenetically the S. Nicolau birds group with the other Cape Verde warbler populations rather than with the greater swamp warbler (Figure 2.2). Additionally, the tMRCA of S. Nicolau and the two Sotavento populations is 199 kya. Consequently, the current S. Nicolau population cannot have been derived from birds that arrived from Santiago or Fogo in the late 20th century. It is also highly unlikely that S. Nicolau was recolonised by greater swamp warblers dispersing directly from Africa after the original population was extinct. There is a strong indication that a bottleneck has occurred in this population, and based on our own sampling effort we estimate a maximum of 20–25 breeding pairs currently on the island. S. Nicolau is known to have suffered from severe droughts during the last century (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986) and this and other factors, such as predation and/or pathogens, may have reduced the warbler population. However, we cannot accurately estimate by how much it has decreased given that there is no reported estimate of its original size, and assessing possible habitat loss is beyond the scope of this paper. Thus, any links between bottlenecks and constraints on population size are only speculative at this point. Clearly, the warbler population on S. Nicolau should be considered to be of high conservation concern.

The estimated tMRCA between the three Cape Verde warbler populations (165–199 Kya) is not that much more recent than the tMRCA between the Cape Verde and greater swamp warblers (292 Kya; Table 2.3), suggesting that there was little gene flow between the Cape Verde warbler populations after the colonisation of the islands. However, divergence dates have an associated error (95% HPD; Table 2.3) and the use of different markers, substitution rates, calibration methods or molecular clocks can lead to variation in estimated tMRCA and divergence times (e.g. Cibois *et al.* 2011; Ho 2007; Yoshikawa *et al.* 2008). Consequently tMRCA must be treated with caution. For example, the molecular substitution rate we used was 2.1% sequence divergence per million years (Weir & Schluter 2008). However, there is evidence that no universal molecular clock rate exists for birds, and that substitution rates can be higher than 2.1% for recently diverged sister species or for intraspecific datasets (García-Moreno 2004; Lovette 2004; Subramanian *et al.* 2009). Nevertheless, it is clear that the Cape Verde warbler diverged from its sister species within the last million years (geometric mean estimate = 292 Kya), as previously suggested (Fregin *et al.* 2009; Hazevoet 1995), and that the three Cape Verde warbler populations have been isolated from each other since before the Last Glacial Maximum (> 30 Kya) and perhaps much longer (199 Kya). That Santiago and Fogo share the same most common cytochrome b haplotype (Figures 2.2 and 2.3), and have a tMRCA estimate of 165 Kya suggest that the split between these two populations was more recent than the split between them and S. Nicolau (199 Kya), with which they share no cytochrome b haplotypes.

Our results indicate that the populations have been through bottlenecks of variable intensity, with particularly strong evidence for the S. Nicolau population (Table 2.6). The M-ratio, which tests for occurrence of gaps in allele size range, can still show a bottleneck signal long after the signal is no longer detectable with heterozygosity or shift mode tests (Garza & Williamson 2001; Peery *et al.* 2012; Williamson-Natesan 2005). It is tempting to infer that, even though all the warbler populations appear to have undergone bottlenecks, the one affecting S. Nicolau occurred more recently, or is still ongoing, while in Fogo and Santiago the populations have stopped decreasing, or are even increasing in size. Contrasting results from M-ratio and heterozygosity tests are commonly used to infer differences in the timing of bottlenecks (Garza & Williamson 2001; Peery *et al.* 2012; Williamson-Natesan 2005), but these tests can be unequally affected if assumptions are violated (Peery *et al.* 2012), so results must be interpreted with caution. Therefore, even though our data show that the S. Nicolau population has been recently, or still is, bottlenecked, and that evidence for bottlenecks in the other two populations is not as strong, this does not exclude the possibility that the other populations are currently decreasing as well.

Despite extensive surveys, we did not find warblers on either Brava or Santo Antão, consistent with results from previous work (H. Dinis & E. Ramos, *pers. comm*.). This means that the Brava population, last reported in 1969 (Hazevoet 1995), is very likely to be extinct. A possible explanation is that, as the second smallest island in the archipelago, it would not have had sufficient suitable habitat to support a population through the drought periods that the archipelago suffered in the 1970s and 1980s (Correia 1996; Hazevoet 1995). In the case of Santo Antão, a lack of historical information means we are unable to make any inferences about the possible presence of a population there in the past.

The three populations of the Cape Verde warbler fall under the definition of MUs, but not under the definition of ESUs. There is no evidence of gene flow among them, they are genetically distinct, but there is sharing of one mitochondrial haplotype at least between Fogo and Santiago. The population of S. Nicolau is especially distinct, as it does not share mitochondrial haplotypes with either of the Sotavento populations, even though they are not reciprocally monophyletic. From a conservation genetics perspective, it may appear prudent to manage all three populations as separate units, such as suggested for similar situations in other taxa (Fouquet *et al.* 2010; Garcia-del-Rey *et al.* 2013; Reynolds *et al.* 2015). However, the decision to preserve each population's genetic uniqueness in the face of future risks of inbreeding depression or a limited adaptive potential is not straightforward (Amos & Balmford 2001; Coleman *et al.* 2013; Miller *et al.* 2009; Weeks *et al.* 2011; Weeks *et al.* 2016). Augmentation of the number of individuals on some islands, to help prevent inbreeding and maximise adaptive potential, may be beneficial to the populations' chances of survival (Weeks *et al.* 2011; Weeks *et al.* 2016). However, such actions could potentially lead to outbreeding depression (Edmands 2007) and the relative potential costs and benefits must therefore be assessed carefully. In this work, we did not investigate potential direct threats to any of the three populations, such as pathogens, predation or habitat destruction; we discuss our findings solely from a conservation genetics perspective.

Conservation conclusions and recommendations

Our genetic data suggest that each of the three remaining populations of the Cape Verde warbler should be treated as different MUs (Moritz 1994b; Ryder 1986), with different conservation priorities (high on S. Nicolau, medium on Fogo, low on Santiago). Special attention should be given to the more genetically divergent (and most genetically depauperate) population on S. Nicolau. Territory occupancy is currently being monitored at several locations on Santiago and Fogo (A. Rendall, J. Mascarenhas, A. Rodrigues, *pers. comm.*). Fewer conservation actions have been directed to S. Nicolau, where monitoring has been irregular over the last few years (L. Oliveira, *pers. comm.*). From previous surveys (Donald *et al.* 2004; Hazevoet *et al.* 1999) and our own sampling effort, we estimate that S. Nicolau currently holds a maximum of 20–25 breeding pairs (minimum 12; pers. obs.) and therefore could be considered to be on the verge of extinction. At the moment, the relative roles of genetic versus ecological factors in constraining population sizes on any of the islands are not clear and further information on this topic is required to help inform conservation actions. It is important to undertake baseline studies on this species (e.g. population sizes estimates, breeding behaviour, productivity, diet, habitat and predation), preferably on all islands as ecological differences might exist between populations. Until such studies are undertaken, managers should incorporate in their plans actions that take this bird's habitat into account. For example, in the Natural Park of Serra Malagueta, not all the invasive shrubs were removed in a recent restoration action because some warblers were nesting there (J. Mascarenhas, *pers. comm*.). Direct conservation actions and the use of augmentation in the population of S. Nicolau should be considered to help conserve this population. We hope that this genetic analysis will be of use to practitioners in refining action plans to maximise the conservation outcome for this endemic species.

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S2.1 Details of sample collection and age determination

Samples were collected in two field seasons. The first one took place from November 2013 to January 2014, immediately after the rainy season (Correia 1996); and the second one from September to November 2014, during the rainy season, when the birds are thought to breed (Hazevoet 1995). Sampling during or immediately after breeding season allows distinguishing fresh juveniles from worn adults caught in the same territory, and thus avoid using related individuals in microsatellite analyses. We determined if the birds were adults or juveniles based on previously described criteria plumage patterns (Cramp & Perrins 1992), feather moult patterns (i.e., juveniles had fresh plumage during the rainy season while adults had ragged plumage and/or were undergoing moult to some extent) and eye colour variation as previously described for other warblers (Komdeur 1991; Gargallo 1992). When age could not be determined, we assumed that there was a reasonable possibility that they were juveniles, and they were removed from the microsatellite analyses if they had been caught in the same territory as an adult. In addition to the samples collected in the field, we used five CVW blood samples collected in central Santiago by Dr. JC Illera in 2010, two samples collected by Dr. M Melo in 2014; one GSW DNA sample, collected by Dr. B Giessing in Senegal in 2007 and provided by Dr. M Haase, six GSW blood samples collected by TC Omotoriogun in Nigeria in 2013 and provided by the Natural History Museum of Oslo; and three SW samples collected in 2009 on Cousin island by the Seychelles Warbler Research group.

Supplementary table S2.1 Details of the Cape Verde warbler blood samples collected. Altitude was measured in metres above sea level, with a Garmin eTrex® H GPS; geographical coordinates were recorded with the same device and are given in WGS 84 system; for age determination, see text.

Sample #	Island	Altitude	Latitude	Longitude	Date	Time	Ring number	Age	Sex
1	Santiago	135	15.07297	-23.55918	24/11/2013	17:50	A000002	Juvenile	Male
2	Santiago	366	15.05090	-23.60833	25/11/2013	08:45	A000005	Adult	Male
3	Santiago	357	15.05092	-23.60945	25/11/2013	14:00	A000011	Adult	Male
4	Santiago	357	15.05092	-23.60945	25/11/2013	14:40	A000012	Juvenile	Female
5	Santiago	366	15.05090	-23.60833	26/11/2013	09:52	A000016	Adult	Male
6	Santiago	358	15.05065	-23.60403	26/11/2013	13:25	A000017	Adult	Male
7	Santiago	358	15.05065	-23.60403	26/11/2013	14:10	A000018	Adult	Female
8	Santiago	398	15.04720	-23.60558	26/11/2013	17:00	A000019	Adult	Female
9	Santiago	366	15.05090	-23.60833	27/11/2013	08:40	A000023	Adult	Female
10	Santiago	452	15.04264	-23.60569	27/11/2013	13:15	A000025	Adult	Male
11	Santiago	398	15.04720	-23.60558	27/11/2013	17:50	A000027	Adult	Male
12	Santiago	22	15.22911	-23.73972	29/11/2013	12:30	A000028	Adult	Male
13	Santiago	21	15.13217	-23.52942	30/11/2013	12:20	A000029	Adult	Male

Supplementary table S2.1

Sample #	Island	Altitude	Latitude	Longitude	Date	Time	Ring number	Age	Sex
14	Santiago	21	14.92108	-23.60074	02/12/2013	10:15	A000030	Juvenile	Female
15	Santiago	30	14.92407	-23.60145	02/12/2013	13:30	A000031	Juvenile	Male
16	Santiago	30	14.92407	-23.60145	02/12/2013	13:30	A000032	Juvenile	Female
17	Santiago	335	15.05243	-23.60762	03/12/2013	11:45	A000034	Juvenile	Female
18	Santiago	335	15.05243	-23.60762	03/12/2013	11:45	A000035	Adult	Female
19	Santiago	335	15.05243	-23.60762	03/12/2013	12:30	A000036	Adult	Male
20	Santiago	323	15.05224	-23.60744	03/12/2013	15:04	A000038	Adult	Male
21	Santiago	323	15.05224	-23.60744	03/12/2013	15:04	A000039	Adult	Female
22	Santiago	323	15.05224	-23.60744	03/12/2013	16:20	A000042	Undetermined	Female
23	Santiago	323	15.05224	-23.60744	03/12/2013	16:35	A000043	Undetermined	Male
24	Santiago	57	14.92825	-23.59861	04/12/2013	11:35	A000045	Adult	Male
25	Santiago	93	14.93402	-23.59910	04/12/2013	15:05	A000046	Juvenile	Female
26	Santiago	93	14.93402	-23.59910	04/12/2013	16:10	A000047	Adult	Female
27	Santiago	93	14.93402	-23.59910	04/12/2013	16:10	A000048	Adult	Male
28	Santiago	336	15.05072	-23.60354	05/12/2013	15:34	A000050	Adult	Male
29	Santiago	336	15.05072	-23.60354	05/12/2013	15:34	A000051	Adult	Female
30	Santiago	310	15.05299	-23.60622	07/12/2013	17:00	A000054	Adult	Male
31	Santiago	310	15.05299	-23.60622	07/12/2013	17:25	A000055	Adult	Female
32	Santiago	138	15.07367	-23.55428	08/12/2013	17:22	A000056	Adult	Male
33	Santiago	138	15.07367	-23.55428	08/12/2013	17:22	A000057	Juvenile	Female
34	Santiago	138	15.07367	-23.55428	08/12/2013	18:15	A000058	Juvenile	Male
35	Santiago	138	15.07367	-23.55428	08/12/2013	18:30	A000059	Adult	Female
36	Santiago	135	15.20632	-23.65713	09/12/2013	12:11	A000061	Adult	Male
37	Santiago	27	15.24336	-23.65592	09/12/2013	17:25	A000062	Adult	Male
38	Santiago	22	15.23974	-23.65415	10/12/2013	12:30	A000063	Adult	Male
39	Santiago	22	15.23974	-23.65415	10/12/2013	13:10	A000065	Adult	Female
40	Santiago	130	15.20888	-23.66544	10/12/2013	16:10	A000066	Adult	Male
41	Santiago	210	15.19793	-23.67876	11/12/2013	09:10	A000067	Adult	Male
42	Santiago	210	15.19793	-23.67876	11/12/2013	09:25	A000068	Adult	Female
43	Santiago	295	15.18919	-23.68171	11/12/2013	11:45	A000070	Adult	Male
44	Santiago	1017	15.18016	-23.67312	12/12/2013	13:22	A000073	Adult	Male
45	Santiago	1048	15.17854	-23.67320	12/12/2013	14:50	A000074	Adult	Male
46	Santiago	903	15.03443	-23.61415	14/12/2013	17:44	A000076	Adult	Male
47	Santiago	1042	15.17875	-23.67276	15/12/2013	15:30	A000077	Adult	Male
48	Santiago	1023	15.18078	-23.67272	16/12/2013	11:25	A000080	Adult	Female
49	Santiago	1019	15.18156	-23.67242	16/12/2013	12:42	A000081	Adult	Male
50	Santiago	1019	15.18156	-23.67242	16/12/2013	13:08	A000082	Adult	Female
51	Fogo	880	15.02021	-24.32742	19/12/2013	15:50	A000085	Adult	Male
52	Fogo	508	15.01898	-24.32777	20/12/2013	12:54	A000086	Adult	Male
53	Fogo	540	15.01835	-24.32909	21/12/2013	09:35	A000087	Adult	Male
54	Fogo	591	15.01718	-24.32908	21/12/2013	13:45	A000088	Adult	Male
55	Fogo	546	15.01866	-24.33018	22/12/2013	12:00	A000089	Adult	Male
56	Fogo	566	15.01795	-24.33060	22/12/2013	14:45	A000090	Adult	Male
57	Fogo	584	15.01762	-24.32099	22/12/2013	16:00	A000091	Adult	Male
58	Fogo	585	15.01753	-24.33090	22/12/2013	17:25	A000093	Adult	Female
59	Fogo	506	15.01967	-24.32881	23/12/2013	09:00	A000094	Adult	Female

Supplementary table S2.1

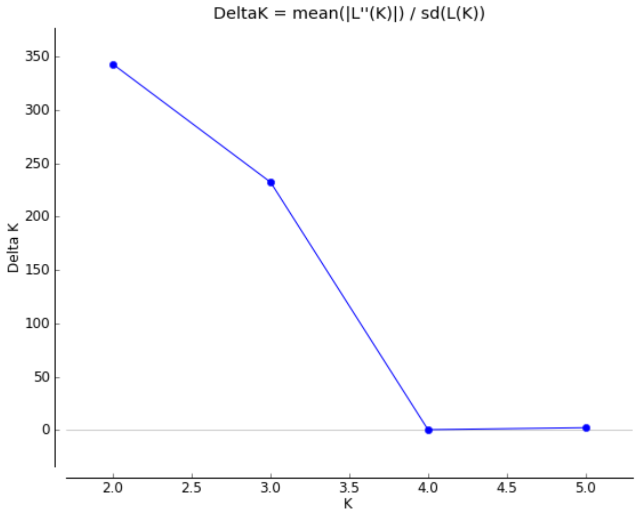
Sample #	Island	Altitude	Latitude	Longitude	Date	Time	Ring number	Age	Sex
60	Fogo	506	15.01967	-24.32881	23/12/2013	09:40	A000095	Adult	Male
61	Fogo	591	15.01687	-24.33145	23/12/2013	12:05	A000096	Undetermined	Male
62	Fogo	591	15.01687	-24.33145	23/12/2013	13:20	A000097	Adult	Female
63	Fogo	612	15.01670	-24.33208	23/12/2013	14:49	A000098	Undetermined	Female
64	Fogo	571	15.01650	-24.33195	23/12/2013	16:40	A000099	Adult	Male
65	Fogo	395	15.02312	-24.33072	26/12/2013	12:05	A000100	Adult	Male
66	Fogo	374	15.02299	-24.32901	27/12/2013	09:27	A000101	Undetermined	Male
67	Fogo	374	15.02299	-24.32901	27/12/2013	09:55	A000102	Juvenile	Female
68	Fogo	405	15.01989	-24.32419	27/12/2013	11:15	A000103	Undetermined	Male
69	Fogo	417	15.01998	-24.32549	27/12/2013	13:10	A000104	Adult	Male
70	Fogo	435	15.01931	-24.32527	27/12/2013	15:15	A000105	Juvenile	Female
71	Fogo	435	15.01931	-24.32527	27/12/2013	15:15	A000106	Undetermined	Male
72	Fogo	379	15.02381	-24.32953	28/12/2013	14:22	A000107	Adult	Male
73	Fogo	379	15.02381	-24.32953	28/12/2013	15:20	A000109	Undetermined	Male
74	Fogo	447	15.01964	-24.32666	29/12/2013	10:00	A000110	Adult	Male
75	Fogo	447	15.01964	-24.32666	29/12/2013	11:14	A000111	Juvenile	Female
76	Fogo	447	15.01964	-24.32666	29/12/2013	12:06	A000112	Adult	Female
77	Fogo	507	15.01776	-24.32670	29/12/2013	14:00	A000114	Adult	Male
78	Fogo	397	15.02026	-24.32497	30/12/2013	08:55	A000116	Adult	Male
79	Fogo	435	15.01558	-24.32261	30/12/2013	12:54	A000117	Adult	Male
80	Fogo	527	15.01495	-24.32501	31/12/2013	10:27	A000118	Adult	Male
81	S. Nicolau	600	16.63766	-24.35239	07/01/2014	09:30	A000119	Adult	Male
82	S. Nicolau	604	16.63783	-24.35187	07/01/2014	11:08	A000121	Adult	Male
83	S. Nicolau	604	16.63783	-24.35187	07/01/2014	11:17	A000122	Juvenile	Female
84	S. Nicolau	836	16.62915	-24.35030	07/01/2014	16:17	A000123	Adult	Male
85	S. Nicolau	654	16.63747	-24.35427	08/01/2014	10:10	A000125	Adult	Male
86	S. Nicolau	654	16.63747	-24.35427	08/01/2014	10:34	A000126	Juvenile	Male
87	S. Nicolau	668	16.63703	-24.35454	08/01/2014	11:35	A000127	Adult	Male
88	S. Nicolau	749	16.62563	-24.33110	09/01/2014	10:22	A000128	Adult	Male
89	S. Nicolau	758	16.62645	-24.33021	09/01/2014	12:40	A000129	Adult	Male
90	S. Nicolau	431	16.63271	-24.32205	11/01/2014	13:28	A000130	Adult	Female
91	S. Nicolau	431	16.63271	-24.32205	11/01/2014	13:19	A000131	Adult	Male
92	S. Nicolau	385	16.63174	-24.31114	14/01/2014	11:34	A000132	Adult	Male
93	S. Nicolau	689	16.63596	-24.35298	16/01/2014	11:54	A000136	Adult	Male
94	S. Nicolau	201	16.64912	-24.36306	17/01/2014	11:00	A000137	Adult	Male
95	S. Nicolau	496	16.64311	-24.34414	18/01/2014	14:01	A000138	Adult	Female
96	Santiago	21	14.92108	-23.60074	27/09/2014	16:13	A000139	Adult	Male
97	Santiago	48	14.92825	-23.59861	30/09/2014	11:37	A000140	Adult	Male
98	Santiago	126	15.07365	-23.55496	01/10/2014	14:34	A000141	Adult	Male
99	Fogo	561	15.01828	-24.33026	06/10/2014	14:10	A000142	Adult	Male
100	Fogo	567	15.02085	-24.33356	07/10/2014	10:17	A000143	Adult	Male
101	Fogo	702	15.01906	-24.33865	07/10/2014	15:05	A000144	Adult	Male
102	Fogo	702	15.01906	-24.33865	07/10/2014	15:32	A000145	Adult	Female
103	Fogo	701	15.02895	-24.36719	15/10/2014	12:10	A000146	Adult	Male
104	Fogo	1384	15.00458	-24.34227	16/10/2014	18:00	A000148	Adult	Male
105	Fogo	1382	15.00452	-24.34289	17/10/2014	10:14	A000149	Adult	Female

Supplementary table S2.1

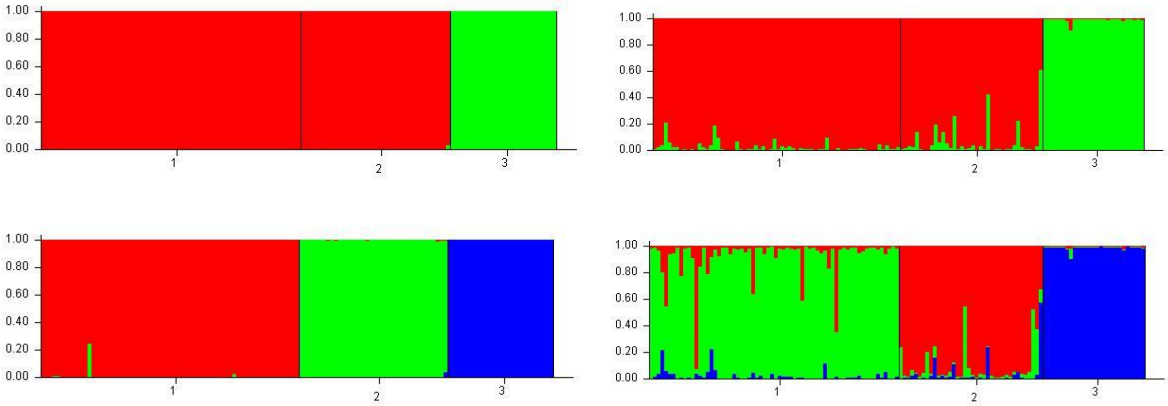
Sample #	Island	Altitude	Latitude	Longitude	Date	Time	Ring number	Age	Sex
106	Fogo	1228	15.00688	-24.34044	17/10/2014	13:07	A000150	Adult	Male
107	Fogo	893	15.01075	-24.33301	18/10/2014	14:28	A000151	Adult	Male
108	Fogo	872	15.01162	-24.33335	18/10/2014	16:25	A000152	Adult	Male
109	Fogo	839	14.99125	-24.42907	20/10/2014	13:32	A000153	Adult	Male
110	Fogo	629	14.99513	-24.31612	24/10/2014	10:14	A000154	Adult	Male
111	Fogo	629	14.99513	-24.31612	24/10/2014	10:30	A000155	Adult	Male
112	Santiago	1039	15.17841	-23.67306	28/10/2014	18:08	A000156	Adult	Male
113	Santiago	1019	15.18383	-23.67085	29/10/2014	10:37	A000157	Adult	Male
114	Santiago	1003	15.17350	-23.67805	29/10/2014	15:55	A000158	Adult	Male
115	Santiago	1043	15.17887	-23.67228	02/11/2014	08:25	A000162	Adult	Male
116	Santiago	1043	15.17887	-23.67228	02/11/2014	08:35	A000163	Adult	Female
117	Santiago	1043	15.17887	-23.67228	02/11/2014	08:35	A000164	Adult	Male
118	Santiago	914	15.03448	-23.61511	07/11/2014	18:05	A000166	Adult	Female
119	Santiago	914	15.03448	-23.61511	07/11/2014	18:05	A000167	Adult	Male
120	Santiago	30	15.11510	-23.52425	09/11/2014	12:22	A000168	Adult	Male
121	Santiago	30	15.11510	-23.52425	09/11/2014	12:26	A000169	Adult	Female
122	Santiago	31	15.11547	-23.52417	09/11/2014	14:42	A000170	Adult	Male
123	Santiago	26	15.12741	-23.76162	12/11/2014	12:04	A000171	Adult	Male
124	S. Nicolau	593	16.63363	-24.34792	17/11/2014	11:15	A000172	Adult	Female
125	S. Nicolau	512	16.64391	-24.34425	18/11/2014	10:45	A000173	Adult	Male
126	S. Nicolau	494	16.65187	-24.34759	20/11/2014	10:55	A000174	Adult	Male
127	S. Nicolau	679	16.62189	-24.33221	21/11/2014	11:48	A000177	Juvenile	Female
128	S. Nicolau	680	16.63199	-24.34981	25/11/2014	09:29	A000178	Adult	Male
129	S. Nicolau	642	16.63123	-24.34816	25/11/2014	12:03	A000179	Adult	Male
130	S. Nicolau	654	16.63347	-24.35071	25/11/2014	13:41	A000180	Adult	Male
131	S. Nicolau	737	16.63307	-24.35244	25/11/2014	15:08	A000181	Adult	Male
132	S. Nicolau	605	16.63782	-24.35182	27/11/2014	13:06	A000182	Adult	Female
133	S. Nicolau	659	16.63647	-24.35346	29/11/2014	11:13	A000183	Undetermined	Female
134	S. Nicolau	659	16.63647	-24.35346	29/11/2014	11:15	A000184	Adult	Male
135	S. Nicolau	689	16.63586	-24.35412	29/11/2014	13:41	A000186	Adult	Female
136	S. Nicolau	670	16.63686	-24.35450	29/11/2014	16:32	A000187	Adult	Female
137	S. Nicolau	516	16.64503	-24.34369	30/11/2014	12:06	A000188	Adult	Male
138	S. Nicolau	516	16.64503	-24.34369	30/11/2014	13:13	A000189	Adult	Female

Supplementary table S2.2 Primer and multiplex details for all microsatellite loci genotyped in the Cape Verde and greater swamp warblers; T_a = annealing temperature.

Primer set	Fluoro-label	Allele size range (bp)	Concentration	Reference
Multiplex 1 (T_a = 55ºC)				
Ase9	ATTO 550	121-144	0.100 µM	Richardson *et al.* (2000)
Ase10	FAM	84-120	0.100 µM	Richardson *et al.* (2000)
Ase37	FAM	227-234	0.200 µM	Richardson *et al.* (2000)
Ase42	ATTO 550	235-241	0.025 µM	Richardson *et al.* (2000)
Ase48	FAM	271-370	0.250 µM	Richardson *et al.* (2000)
Ase58	HEX	224-294	0.200 µM	Richardson *et al.* (2000)
Multiplex 2 (T_a = 55ºC)				
Ase6	FAM	117-173	0.050 µM	Richardson et al. (2000)
Ase13	HEX	120-160	0.001 µM	Richardson et al. (2000)
Ase18	ATTO 550	169-189	0.050 µM	Richardson et al. (2000)
Ase35	HEX	227-231	0.050 µM	Richardson et al. (2000)
Ase56	FAM	293-316	0.200 µM	Richardson et al. (2000)
Multiplex 3 (T_a = 56ºC)				
Ase3	HEX	79-96	0.040 µM	Richardson et al. (2000)
Ase7	FAM	100-109	0.025 µM	Richardson et al. (2000)
Ase11	HEX	109-125	0.025 µM	Richardson et al. (2000)
Ase22	FAM	170-179	0.025 µM	(Richardson et al. 2000)
Cuµ4-Gga5	HEX	235-239	0.050 µM	(Martín-Gálvez et al. 2009)
PmaTGA42	FAM	258-278	0.050 µM	(Saladin et al. 2003)



Supplementary figure S2.1 Graph generated by STRUCTURE HARVESTER (Earl & vonHoldt 2012), displaying the change in ΔK against number of clusters (K) calculated following the method of Evanno et al. (2005), highlighting that K = 2 is the most likely number of genetic clusters, followed closely by k = 3, across the three island populations of the Cape Verde warbler.



Supplementary figure S2.2 STRUCTURE plot (Pritchard et al. 2000) of genetic clustering across the three Cape Verde warbler populations for k = 2 (upper panel) and k = 3 (lower panel), using no-admixture, independent allele frequencies and information on the population of origin (left) or no priors (right). Populations: 1 = Santiago, n= 66; 2 = Fogo, n= ; 38 = S. Nicolau, n = 27.

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Chapter 3

Diversity and divergence in song in the three populations of Cape Verde warbler *Acrocephalus brevipennis*

Male Cape Verde warbler Acrocephalus brevipennis*, photographed after responding to a playback test on Fogo, April 2016.*

3.1 Abstract

Divergence in traits involved in sexual selection can promote barriers to gene flow among populations and thus rapid divergence and, potentially, speciation. In oscine passerine birds, song is, at least partially, a culturally learned trait involved in sexual selection which may drive or reinforce divergence between populations. I tested whether divergence in song exists between the three populations of an endangered island passerine, the Cape Verde warbler *Acrocephalus brevipennis,* and investigated if song may play a role in reinforcing population isolation. I compared song traits from a sample of adult males from each of the three populations. I also created stereotyped songs and conducted playback tests to measure the behavioural responses of territory holders within each island population to songs of males from their own and other populations. I found that, while there is large intra-population and intra-individual variability in song, individuals do not discriminate between the songs of different populations, or even playback songs from different species. In this species song appears to be complex and unpredictable: although males from all populations display different song repertoires they all react equally to a wide range of aural territorial challenges. This suggests that, in Cape Verde warblers, song is not a strong cue for individual or population differentiation. Using male behavioural response as a proxy for female preferences, no evidence was found that song would act to reinforce reproductive isolation between the three diverging populations of this species. However, male reaction to playbacks does not always reflect female perception, thus results should be interpreted with caution.

3.2 Introduction

Archipelagos have proven excellent natural laboratories in which to study evolution and speciation (MacArthur & Wilson 1967; Warren *et al.* 2014). This is because islands are spatially isolated from each other and from the mainland, and dispersal between them can be limited (e.g. Cibois *et al.* 2008; Komdeur *et al.* 2004). Such spatial isolation provides an opportunity for evolutionary forces to act at a population scale (Allendorf & Luikart 2007; Frankham *et al.* 2009; Price 2008). If isolation is extreme, populations may undergo rapid allopatric speciation (Allendorf & Luikart 2007; Frankham *et al.* 2009; Price 2008); that is, stochasticity over time reinforces divergence even when individuals from the different populations do come into secondary contact (e.g. Baker 1959; Cook 1906; Futuyma 2005; Grant & Grant 2009; Irwin *et al.* 2001). The specific drivers of divergence in allopatric populations that lead to speciation are difficult to assess, because they cannot be studied in the wild and must instead be inferred from genetic and phenotypic traits (Helbig *et al.* 2002; Lifjeld *et al.* 2016; Zink 2006).

Reproductive isolation is often dependent on prezygotic mechanisms (Coyne & Orr 2004; Funk *et al.* 2006; Price 2008). Prezygotic isolating mechanisms are those that occur prior to fertilisation, and in animals they can be behavioural, ecological or mechanical (Orr & Smith 1998; Price 2008; Schluter 2001). Divergence in traits involved in mate choice, such as ornamentation and behavioural displays in birds, can play a central role in speciation (Edwards *et al.* 2005; Price 2008). Importantly, song divergence has been implicated in reproductive isolation (Edwards *et al.* 2005; Grant & Grant 2002; Grant & Grant 2009; Irwin *et al.* 2001; Toews & Irwin 2008).

In birds, sympatric speciation is rare (Edwards et al. 2005; Price 2008). For sympatric speciation to occur, two populations must diverge in one or more traits in order to avoid hybridisation. In birds, this often implies divergence in ecological or behavioural traits such as habitat preferences, time of breeding or song (reviewed in Price 2008). For example, in song sparrows *Melospiza melodia*, two sympatric subspecies differ both in song traits and habitat, as a possible results of assortative mating, and both males and females reply more strongly to songs of their own subspecies, suggesting that song acts to reinforce pre-mating reproductive barriers between these two subspecies (Patten *et al.* 2004). It is often assumed that the same traits will diverge in allopatric populations, simply because of drift, and that this results in reproductive isolation upon secondary contact (Edwards *et al.* 2005; Grant & Grant 2002). A classic example of song divergence in allopatric populations is that of the greenish warbler

Phylloscopus trochiloides (Irwin *et al.* 2001), where divergence in song and mitochondrial DNA in neighbouring populations results in reproductive isolation at the two ends of the species distribution, in the shape of a ring around the Tibetan plateau. However, genetic divergence does not always accompany behavioural or trait-based divergence (Perera *et al.* 2007; Phillimore *et al.* 2008). Secondary contact hybridisation can occur between populations that diverged in allopatry but later come into sympatry could occur (Price 2008; Slabbekoorn & Smith 2002). This is possible if phenotypic reproductive barriers are weak, sometimes even in spite of putatively useful ecological adaptations (Grant & Grant 1989; Grant & Grant 1992). Examples abound of contact zones among *Acrocephalus* and *Hippolais* warblers (e.g. Hansson *et al.* 2003; Hansson *et al.* 2012; Lemaire 1977; Secondi *et al.* 2003). When reproductive isolation between allopatric populations is complete there can be character divergence or convergence in sympatry (Haavie *et al.* 2004; Tobias & Seddon 2009), and this relationship can be asymmetric. In sympatric pied flycatchers, songs are more similar to those of collared flycatchers than in allopatric populations, while the opposite is true for collared flycatchers (Haavie *et al.* 2004).

Bird song is involved in mate attraction, territory defence, mate stimulation (Catchpole 1983; Catchpole & Slater 2008) and even prenatal thermal acclimatisation (Mariette & Buchanan 2016). Natural, sexual and cultural selection on bird song can lead to the evolution of differences between populations (reviewed in Podos et al. 2004; Price 1998). As a commonly sexually selected trait, it is thought to be used by females as a cue to assess male quality (Catchpole 2000; Hasselquist *et al.* 1996; Parker *et al.* 2006). Therefore, songs and repertoires can be differently selected; for example, in some bird species females are known to favour males with a larger repertoire (Byers & Kroodsma 2009; Catchpole 1980). Often female birds react more strongly to songs from males from their own population than from other populations, including neighbouring populations (Harris & Lemon 1976; Toews & Irwin 2008), or between their mates and strangers (Seddon & Tobias 2010) indicating that differences in song, even across small spatial scales, are quite common and may have selection-relevant consequences, e.g. differences in fitness (Baker 1983; de Boer *et al.* 2016; Mortega *et al.* 2014). Cultural selection, on the other hand, is the differential copying of songs by individuals in one population based on their effectiveness as communication signs, but not on the fitness of the singing bird (Podos *et al.* 2004). Stochastic factors can also cause songs to diverge, as for example genetic drift which can potentially leading to reproductive isolation even before genetic divergence would suggest that isolation is complete (Irwin et al. 2001; Toews & Irwin 2008). Additionally, cultural drift, i.e. changes in song driven by random variation in their propagation across generations, has been hypothesised as a driver of song change during island colonisation (Podos *et al.* 2004). Song-mediated reproductive isolation mechanisms may play an important role in speciation in some bird species (Grant & Grant 2002; Irwin *et al.* 2001).

There are several mechanisms by which differences between songs of different populations can arise. Bird song contains innate and learned components (Thorpe 1958), and juvenile oscine passerine birds learn to sing from parents and neighbours (Thorpe 1958; Waser & Marler 1977). Juveniles start to copy adult birds songs, but during the first year of life each individual's song is not necessarily consistent, and this phase is called the plastic song (Nottebohm 1968, 1969). At this stage changes in the learned song can occur: some learned syllable types can be dropped, new syllables can be improvised and other unusual syllable types can be retained, making this the phase in which cultural mutations in bird song can occur (Nottebohm 1968, 1969). After this stage, the song can become rather stereotyped, i.e. uniform within each individual and between individuals, and mutations or improvisations no longer occur (Nottebohm 1969; Thorpe 1958; Waser & Marler 1977). Because it is culturally transmitted, bird song can evolve faster than morphology or genetics (Price 2008). It is thus conceivable that opportunities for selection based on song divergence should be commonplace and lead to a behavioural barrier to reproduction between individuals from two populations (Price 2008).

If bird populations are reproductively isolated, both males and females are predicted to respond more strongly to the songs of their own population than to songs of other populations or species (Grant & Grant 2002; Mortega *et al.* 2014; Searcy *et al.* 1997). Song playback experiments are a useful tool to assess this (Grant & Grant 2002; Mortega *et al.* 2014). They are used to elicit responses, ideally in receptive female birds and usually in experimental aviary contexts (Baker 1983; Catchpole *et al.* 1984; Hoelzel 1986). There are problems with testing female responses to male song in the wild, as they may not be reproductively receptive at the given point of the experiment, or might be inhibited by the presence of a dominant male (Catchpole *et al.* 1984; Grant & Grant 2002). Thus, in typical field-test playbacks the responses of the focal territorial male to an intruder are often used as a proxy of likely female response (Grant & Grant 2002). However, this approach has limitations because males and females can react differently to playback tests (Seddon & Tobias 2010). For example, in playback tests conducted in antbirds, *Hypocnemis peruviana* males reacted aggressively to conspecific and heterospecific songs in sympatric areas, but females were able to discriminate between conspecifics and heterospecifics and even between their mates and conspecific strangers (Seddon & Tobias 2010; Tobias & Seddon 2009). Thus caution must be used when inferring female preferences from males behavioural responses to playback tests. In additional factor to consider in playback tests is the function of the song elements used. For example, bird song is usually linked to mating and breeding, while bird calls are often used to signal alarm or other circumstances non related to mating (Marler & Slabbekoorn 2004; Slabbekoorn & Smith 2002).

The Cape Verde warbler *Acrocephalus brevipennis* is a small warbler (14–20 g) which inhabits dense vegetation (Hazevoet 1995) and is endemic to the Cape Verde archipelago ca. 500 km off West Africa (Figure 3.1). This bird is a resident species on the islands of Santiago, Fogo and S. Nicolau (Cramp & Perrins 1992; Garcia-del-Rey 2016) and is currently classified as 'Endangered' (BirdLife International 2016). The three populations of the warbler have been isolated from each other since at least 165 kya, and they are genetically divergent, particularly the small isolated population on S. Nicolau (Batalha *et al.* 2017). It is not currently known if these populations constitute different taxonomic entities or are reproductively isolated. The Cape Verde warbler's song is said to be similar to that of its sister species (Cramp & Perrins 1992), the greater swamp warbler *Acrocephalus rufescens* (GSW) of sub-Saharan Africa (Fregin *et al.* 2009; Leisler *et al.* 1997). However, no formal studies have ever tested this, possibly because of low sample sizes. Preliminary observations indicated that there is considerable inter- and intra-individual variation in bird song within each island (Cramp & Perrins 1992; pers. obs.).

Here, I aim to assess divergence in song to understand the degree to which the three populations of Cape Verde warbler might be reproductively isolated. I aimed to 1) assess divergence in song traits between populations, and 2) use behavioural response differences to assess the reproductive relevance of any existing divergence. Given that there is no evidence of gene flow among the islands (Batalha *et al.* 2017), I predicted that there will be significant differences between islands, both in song traits and behavioural reaction to songs of birds from the other islands. However, if inter-individual variation in bird song is high and not correlated with isolation, I predict that the birds are likely to perceive songs from all populations as belonging to novel individuals and react similarly to all songs.

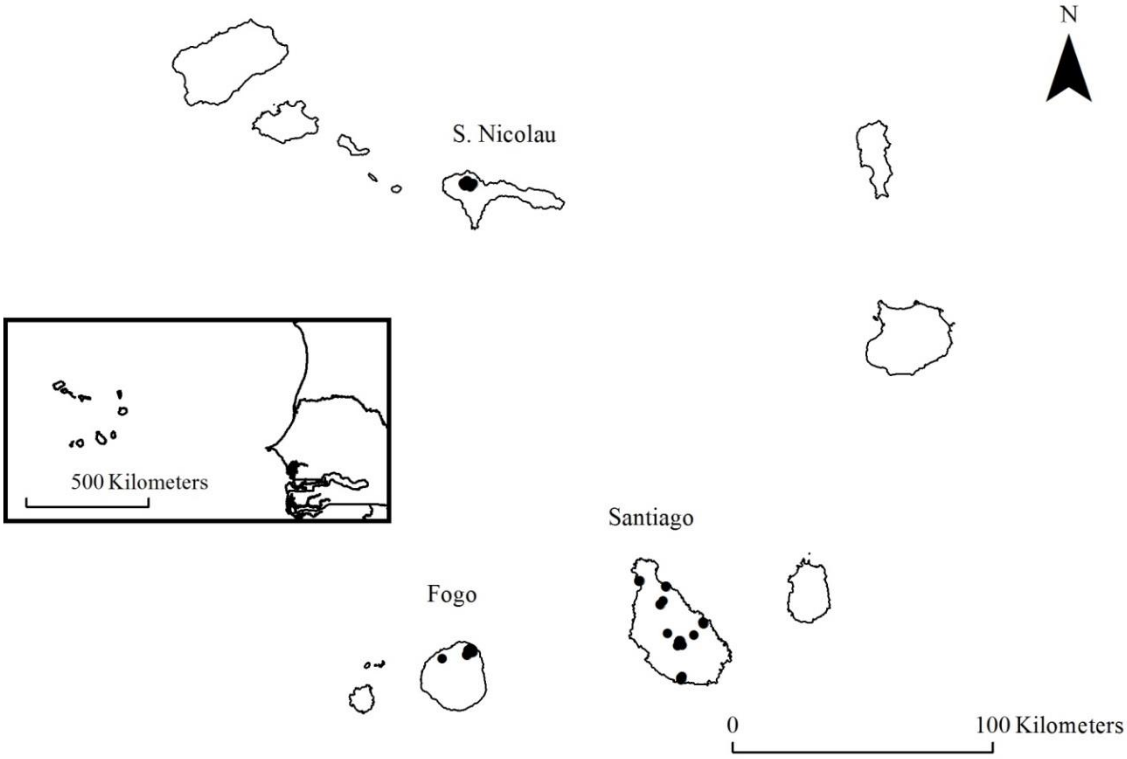


Figure 3.1 Map of the Cape Verde islands (main) with position relative to West Africa (inset). Cape Verde warblers exist on Santiago, Fogo and S. Nicolau. Locations where male songs were recorded and/or playback tests were conducted between 2014 and 2016 are shown in black circles (see Figures S3.3 and S3.4 for more detail). *Map produced using a combination of open source and original data on ArcMap 10.1 by Helena Batalha.*

3.3 Materials & Methods

3.3.1 Song characterisation

The Cape Verde warbler is thought to breed after the first rains, i.e. usually between August and November (Hazevoet 1995). However, males are thought to be territorial throughout the year (Cramp & Perrins 1992; Hazevoet 1995; Hazevoet *et al.* 1999) and there is anecdotal evidence that pair-bonding takes place during the dry season, i.e. in April–May, and the warblers are heard singing year-round (A. Rendall, pers. comm.; Cramp & Perrins 1992). I recorded songs and calls from males from September 2014 to November 2014 (hereafter 2014), and conducted playback tests from April to May 2016 (hereafter 2016). I surveyed for warblers extensively across different habitat types and altitudes (up to 1384 m) on each of the three islands to ensure the sampling was as comprehensive as possible. I did not conduct traditional transects or point counts to locate territories, for two reasons. First, because the Cape Verde warbler sings very irregularly throughout the day and can hold large territories (pers. obs.), which might cause it to be silent or in some other part of its territory in the moment when a song is played back, thus not responding to it even though it is present in the area (Batalha 2014; Donald *et al.* 2004). Second, patches of vegetation that could potentially be occupied by the warbler appear rather irregularly throughout the Cape Verde islands (pers. obs.), and this can cause traditional censuses or regularly spaced point counts to fall outside potentially suitable habitat or in inaccessible locations (Batalha 2014). For these reasons, traditional census methods can miss many individuals (Batalha 2014; Donald *et al.* 2004), and were not used. Previous observations had shown that the Cape Verde warbler inhabits densely vegetated patches on all three islands (Donald *et al.* 2004; Hazevoet 1995; Hering & Fuchs 2009). Initial surveys confirmed that the warbler was absent from more barren areas. I then restricted my searches to areas with structured vegetation (of any kind), including areas where the bird had been previously detected (Batalha *et al.* 2017; Donald *et al.* 2004; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Fuchs 2009), and areas with dense vegetation but where the bird had not been recorded before. I conducted transects and used playbacks whenever I was crossing or close to patches of potentially suitable habitat. If male Cape Verde warbler responded in the vicinity, I would get closer and try to determine the territory used by the warbler. This was done in as many different areas of each island as possible when taking into account logistic and time constraints. This means that the sampling was not completely random, thus artificially increasing the probability of finding the birds. However, the alternative would be to risk not obtaining a large enough sample size within the restricted time frame available.

After locating Cape Verde warblers, recordings were made with a Sennheiser ME66 microphone coupled to a Sony IC Recorder ICD-PX333 (stereo, sample rate 44.1 kHz, resolution 32-bit float). In 2014, recordings were made before or after, but not during, playback used to attract the birds. In 2016, recordings were made during the playback tests. In both years, the songs were all recorded in MP3 format, which compresses the sounds, but as the Cape Verde warblers sing within a frequency range of 1000 to 5000 Hz (Table S3.3) compression would not be a problem. To attract birds in 2014 I used playback through X-mini II Capsule speakers, model Xam 14, with an output of 2.5W, frequency response 100 Hz to 20 kHz, signal-to-noise ratio ≥80 db and distortion ≤1%. In 2016, I used Arespark AS200-BK speakers, with a total output of 7W, frequency response 68 Hz to 20 kHz, signal-to-noise ratio and distortion unknown. All songs were analysed with Audacity 2.0.5 and Raven Pro version 1.4 (Cornell Lab of Ornithology, USA).

To examine the repertoire of Cape Verde warblers, I first visually categorised each individual's song spectrograms into *song types*, and within those unique *variations*. I defined *song type* as an assemblage of unique motifs/syllables/elements; a *variation* was defined as the inclusion, removal or reordering of motifs/syllables/elements within a song type (Figure 3.2).I assessed repertoire size for the Cape Verde warbler by classifying and counting the number of different song types and variations within a song type (Table S3.1). I used only songs recorded in 2014 from colour-ringed males or males recorded in known locations, i.e. songs that could be attributed to known males. I visually inspected the maximum consecutive number of songs possible per individual male but this ranged from four to 32 (Supplementary table S3.1) and I caution against drawing inferences from such small sample sizes.

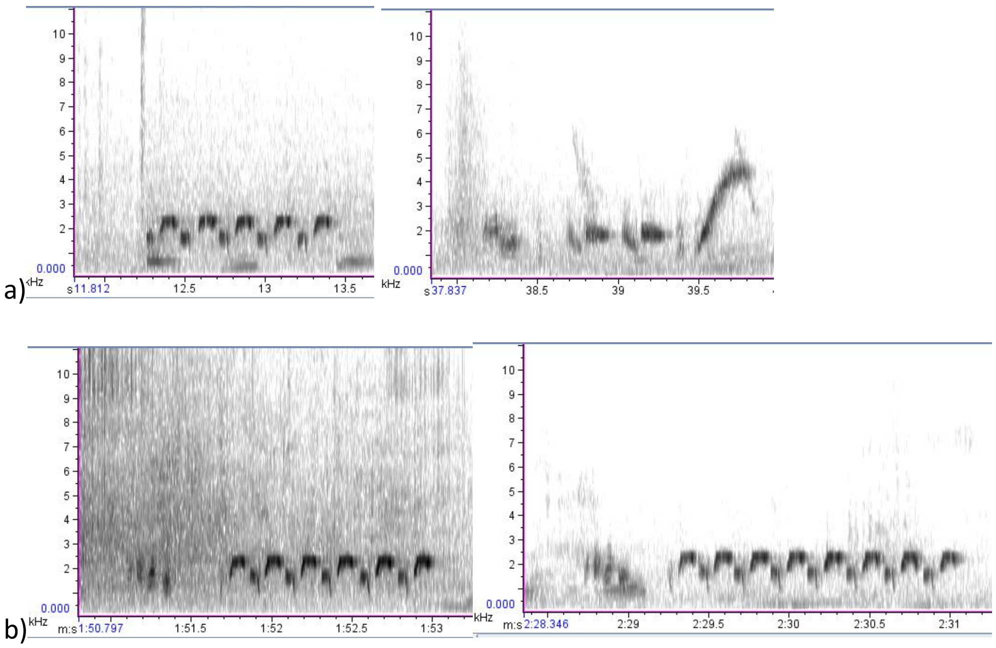


Figure 3.2 Example of song types and variations within song types in the Cape Verde warbler; a) two *song types* of one Santiago bird: the elements composing each song type are different; b) two *variations* within a song type of one Fogo bird: all or most of the elements are the same, but the number of repetitions and/or order in which they are arranged changes; in other cases there can be inclusion or removal of elements. See full repertoire size in Table S3.1. *Figures produced using original data on Raven Pro 1.4 by Helena Batalha.*

I used 2014 recordings to analyse spectrograms of songs of different males on each island. I visualised the spectrograms in Audacity, converted stereo tracks into mono, removed background noise when necessary (up to 15 dB) and exported the track as a wav file. If the recording had been made while to bird was replying to a playback, we only analysed spectrograms sung by the bird and discarded the recordings of playbacks. I calculated the song rate by counting the number of bird songs in each recording. The wav file was analysed with

Raven Pro version 1.4 (Bioacoustics Research Program 2011), where I selected only clear and distinguishable song spectrograms, with no overlapping songs or other strong background noise. I generated spectrograms keeping the pre-defined viewing settings (Bioacoustics Research Program 2011). I drew selection boxes around the song spectrograms, excluding the calls that preceded each song, and measured *aggregated entropy* (u), *average entropy* (u), *first quartile (Q1) frequency* (Hz), *third quartile (Q3) frequency* (Hz), *centre frequency* (Hz), *5% frequency* (Hz), *95% frequency* (Hz) and *delta time* (s). Entropy measures have been used as proxies of vocal complexity, especially in case where full measures of repertoire size are unattainable due to small sample size (e.g. Da Silva *et al.* 2000; Kershenbaum 2014), and have been successfully used in other animal vocalisation studies (Fernández-Vargas & Johnston 2015). Average entropy measures the disorder of the energy within a subsection (a 'slice') of the sound in the selection box (Charif *et al.* 2010). Thus, more pure tones will have lower entropy values than higher amplitude sounds. Aggregated entropy measures the disorder of the energy within the entire selection box (Charif *et al.* 2010); that is, more monotonous or repetitive songs will have lower entropy values than more complex or polytonal songs. Entropy-based measurements (aggregated entropy and average entropy) take into account all the sounds inside the selection box and thus can be affected by background noise, so I only used spectrograms that had little or no background noise. Frequency measurements quantify the frequency below which a given portion of the total energy within the selection box lies. The frequency-based measurements I selected (first quartile frequency, third quartile frequency, centre frequency, 5% frequency and 95% frequency) are all robust to the size of selection boxes and low energy background noise (Charif *et al.* 2010). I confirmed this in two ways. First, I compared the measurements of different songs made up of the same elements. Second, I measured the same song several times with different selection boxes. Delta time is the difference, in seconds, between the left and right limits of the selection box, which is manually selected by the user. Songs are frequently, but not always, preceded by one or two calls. These were not included in the selection boxes because both the number of calls and the pause length between each call and the subsequent song varied greatly (e.g. from 0.15 to 0.70 seconds; data not shown but see supplementary material 2), which could introduce undesired noise.

3.3.2 Playback tests

On a typical playback test, birds from a given population are exposed to songs from their own population, an allopatric population and a control population or species, and the strength of their response to each song is quantified by measuring reactions in the field (e.g. Grant & Grant 2002; Mortega *et al.* 2014). To ensure the birds are reacting to the songs of one population and not one individual, and to avoid pseudo-replication, one different song per individual test is ideally used (reviewed in Kroodsma 1989; Kroodsma 1990). Due to logistic impediments or lack of spontaneous recordings to begin with, this is not always possible, but in that case experiments must control for pseudo-replication in the data analysis (Kroodsma 1989; Kroodsma 1990).

I tested for the differences in the birds' reaction to playbacks from five different origins: each of the three warbler populations (Santiago, Fogo and S. Nicolau), the sister species GSW and a control species (the Seychelles warbler, SW, *Acrocephalus sechellensis*). I adapted the playback protocol used by Mortega *et al.* (2014). Tests were conducted from 21 April to 7 May 2016, during part of what is thought to be the pair-bonding period (A. Rendall, pers. comm.; Cramp & Perrins 1992). To create Cape Verde warbler playback songs, I used individual recordings made in 2014, from a minimum of 5 different males per island. These songs were sung during the breeding season either spontaneously or in response to playbacks of birds of their own population. The identity and/or location of the males providing the songs were known (Figure S3.3). There is apparently high variability in syllables, song types and song rate between and within individuals on each island (Table 3.1 and S3.1). Consequently I was unable to determine the most common song type on each island (see Bell *et al.* 2004) to build standardised population songs (as in Mortega *et al.* 2014). The use of only one song type repeated for the duration of the playback song would have been unnatural to the birds and would not represent a natural male intruder. To mimic natural variability in the playback experiment, I built each playback song by selecting segments of a recording from a given male, including from one to six consecutive songs, or songs and calls, with clear, uncluttered sound profiles which were free from overlap with other birds' songs or strong ambient noises. I reduced the remaining ambient noise in Audacity by 5 to 15 dB, depending on the overall signal-to-noise ratio, converted all the segments between songs into silence, and pasted the segment, maintaining the original pause lengths. From each collage of segments of a given male I generated a 150-second loop, filtered the lower 500 Hz sounds with a 48 dB high-pass filter (this is the strongest filter, but it did not affect the quality of the songs), and amplified each constructed song to a peak of -1 dB of maximum amplification. I repeated the process, using songs from a single male for each collage, until I had at least ten different 150-second playbacks per island. For the other species used in the tests (see below) I applied the same methodology but I used recordings downloaded from Xeno-Canto.org or supplied by the Seychelles Warbler Research Group.

I ran preliminary trials to determine the optimal length of time between playing each song and which measurements could be reliably taken given the habitat structure and the birds' behaviour. I also ran a preliminary test to check the birds' reaction to songs of different warbler species. The goal was to select a control species (i.e. a species to whose song males did not react strongly) and a species that could be used to locate Cape Verde warbler territories (i.e. a species to whose song males reacted but which would not be used in the actual playback tests). I could not use the warbler's own song to locate the territories, as exposing them to a species/population that would later be used in the actual test could bias the reactions (Grant & Grant 2002; Mortega *et al.* 2014). I chose species with songs similar to the Cape Verde warbler in note structure and/or frequency range, or phylogenetically close but geographically distant, which means they had not been heard by the warblers (Grant & Grant 2002; Mortega *et al.* 2014), not even from potential vagrants. I played songs of the Australian reed warbler (ARW, *A. australis*), clamorous reed warbler (CRW, *A. stentoreus*), Madagascar swamp warbler (MSW, *A. newtoni*), lesser swamp warbler (LSW, *A. gracilirostris*), Seychelles warbler (SW, *A. sechellensis*) and common bulbul (CB, *Pycnonotus barbatus*). The pair of warblers tested reacted to all of the songs to some degree, but they reacted less to the MSW, ARW and SW. Therefore, I decided to use the LSW's song to locate territories, and the SW as a control in the playback tests.

To determine which vegetation structures were used by male warblers within a territory, I played the LSW songs at a minimum of 3 different points and noted which structures an individual landed on. This also helped determine which bird was the dominant male, as dominant males tend to sing and approach the speaker more (pers. obs.). I only followed the dominant male, and any tests where the focal male was not clearly the dominant individual throughout the experiment were not included in further analyses. An Arespark S200 speaker was placed in one of the used vegetation structures, at a height that the birds had used or could potentially use. I selected an area with the best possible visibility and ensured there was vegetation used by the bird in a radius at least 5 m around the speaker and covering at least half of the circle within those 5 m. The speaker was connected to an mp3 player by an audio cable, which I operated from a location from where I could observe the birds' movements (Figure 3.3).

I waited 15 minutes between the end of the playback used to locate the structures, and the beginning of the playback test. The speaker location was recorded with a Garmin eTrex® H GPS and a horizontal circular boundary line 5 m from the speaker was marked out. Neither territory mapping playback nor the marking of territorial features appeared to affect the behaviour of warblers (pers. obs.). I then played each of the five test songs in a previously defined randomised order. Because playback songs were individually identified, I never played songs from the same bird I were testing or one of their neighbours, eliminating effects due to host familiarity. No birds were tested twice and I did not test birds in neighbouring territories where the sound could be heard from one territory to the next. However, on S. Nicolau, where warblers are rare (Donald *et al.* 2004; Hazevoet *et al.* 1999) and the territories are concentrated in certain areas, I tested neighbour birds once, but left a three-day interval between the tests. For each test and group, a different song was played, within islands, to avoid pseudo-replication, except on a couple of occasions (Kroodsma 1990). In each test, I played each song for the full 150 seconds duration. During this period of time I recorded:

a) latency to approach within 5 m of the speaker, in seconds, measured with a timer;
b) time spent within 5 m of the speaker in seconds, measured with a timer;
c) number of calls given by the responding bird;
d) number of songs given by the responding bird;
e) minimum distance to the speaker, in approximate metres (this distance was measured in absolute terms, rather than on a plan, because sometimes the birds would land exactly above the speaker, but a couple of metres high, to take vertical distance into account; the distance was estimated to the nearest metre).

Because the vegetation used by the Cape Verde warbler is very dense, it was not possible to record other behaviours such as tail- or wing-flipping and overall number of flights. After each song I waited a minimum of 150 seconds and a maximum of 900 seconds before playing the next song. The goal was to ensure the bird had stopped being reactive and left the area before each of the following songs. I considered that the birds stopped reacting when they 1) moved away more than 10 m from the speaker or 2) were silent for more than 30 seconds after the initial 150 seconds. When one of the two conditions was met we resumed the test. If, after waiting 15 minutes, the bird was still singing/calling or within 10 m of the speaker, I would proceed with the following test, but did not measure latency to approach or time spent within 5 m.

Figure 3.3 Example of a typical playback test on a male Cape Verde warbler *Acrocephalus brevipennis*: the remotely operated speaker is placed in a plant and at a height that the individual would naturally use, most of the 5 m radius around it is covered in said vegetation, and there are three observers following the individual's movements. The red circle shows a dominant male Cape Verde warbler flying above the speaker in response to a playback. *Photo taken on S. Nicolau, May 2016.*

3.3.3 Statistical analysis

Unless stated otherwise, statistical analyses were performed in R v. 2.14.1 (R Core Team 2016) and each island was tested separately. I first determined if spectrogram-based measurements could be reliably used to test for differences between populations. To do this, I assessed if the frequency, entropy and time measurements could be reliably used to discriminate between different song types. I selected spectrograms of 14 song types recorded in the 2016 playback tests: six from Santiago, four from Fogo and four from S. Nicolau (Figure S3.1). This was the minimum number of different song types per island for which I had at least nine spectrograms that I could measure (ranging from nine to 28 spectrograms per song type). I measured the traits described in section 3.3.1, which are robust to background noise within a spectrogram. However, they can be affected by the distance to microphone (e.g. at larger distances, higher-frequency sounds are recorded with more energy than low-frequency sounds) or ambient noise, and it is not possible to control for this in the field. I could not control for distance to microphone but we controlled for the effect of ambient noise by using only spectrograms clear from background sounds. Using absolute or logarithmic values to model frequencies does not qualitatively alter the results (Cardoso 2013); therefore, we used non-transformed variables in all tests. I ran a principal components analysis (PCA) with function princomp, package stats (R Core Team 2016) including all the measured variables. I tested for differences between song types on the first principal component (PC1) with an ANOVA with function aov, package stats. Additionally, I performed a linear discriminant analysis (LDA) to classify spectrogram types with functions vif, package car (Fox & Weisberg 2011) and lda, package MASS (Venables & Ripley 2002). The LDA is a multivariate classification technique which tries to find functions that better explain differences between pre-defined groups. Therefore, it is useful to verify if each spectrogram can be accurately classified as belonging to a certain song type based on its measurements (e.g. Domínguez *et al.* 2016; Illera *et al.* 2014; Palmero *et al.* 2012). It is fairly robust to violation of normality assumptions, but it requires that there is low multicollinearity between the variables. The LDA was followed by a MANOVA to test for differences between groups, performed with function Wilks.test, package rrcov (Todorov & Filzmoser 2009). Wilks' lambda is a test based on the MANOVA table which measures the amount of variance that is not attributable to the grouping factor, i.e. the within-group variance; in other words, the smaller its value, the more of the variance is explained by the grouping factor.

Song differences between populations

I selected different recordings per island, made while males were singing spontaneously during the breeding season of 2014, and I measured spectrograms as described above. Since the goal of this analysis was to test for differences between the three populations, spectrograms of songs from the three islands were pooled together. I ran a similar analysis to the one used to discriminate between song types, but since I measured more than one spectrogram per bird, birds within islands were included as random effects in the ANOVAs. I tested for differences between islands and between individuals. Similarly, for the LDA and MANOVA I first used the island where each spectrogram was recorded as the grouping factor, and then the bird that sang it.

Differences in song response to playback

I tested for differences in the birds' vocal response to playback songs of different origins in 2016 by performing a PCA to extract variance from song spectrogram traits. I then used linear mixed models with function lmer, package lmerTest (Kuznetsova *et al.* 2016), on PC1 to assess if spectrograms would differ depending on the playback song origin that the birds were reacting to, following Mortega *et al.* (2014) and using the rationale behind reference studies (Catchpole 1977; Grant & Grant 2002). I defined spectrograms sung in response to songs from the birds' own population to be the intercept to which the other spectrograms were compared, and used the population of origin of the song to which the birds were responding as a factor. This allowed comparing the responses to each different population/species with the response of individuals to songs from their own population. I included subjects within playback tests as nested random effects to control for repeated measures (each bird would sing several times in response to each test), and trial order. Recordings of songs sung in response to the playback of the LSW – used to assess the occupied habitat area – were also included in the model.

Differences in behavioural response to playback

I examined if birds behaved differently when reacting to songs of different population origin. I assessed correlations between related behavioural measures with spearman.test, package Hmisc (Harrell *et al.* 2016). I then tested the effect of the five different song origins on: 1) latency to approach within 5 m, 2) minimum distance to speaker, and 3) number of songs given by the responding bird. The sample size was limited because of time constraints and population numbers: I had 12 data points per factor level of the independent variable per population. In other words, I tested 12 subjects for each of the five playback tests per island (Figure S3.4). To avoid over-parameterising the models date or time were not included. When included, date and time did not have a significant effect in the behavioural responses of the birds, except date on the number of songs on S. Nicolau (estimate = -0.475, SE = 0.219, Z-value = -2.168, p = 0.03). However, this effect was not consistent across islands or across behavioural tests on S. Nicolau. Latency to approach was analysed using Cox proportional hazard models fitted by maximum likelihood with function coxme, package coxme (Therneau 2015). Minimum distance to speaker and number of songs were modelled with a linear mixed model fitted by maximum likelihood. Again, subjects were included as random effects to control for repeated measures (because in all tests the same subject was measured five times, one per playback test). I included trial order and song rate in every model as fixed effects. The model including the variables of interest was compared with the simplest model, including just the independent variable and the random effect, using ANOVA and AIC (functions anova and AIC, package stats).

3.4 Results

3.4.1 Song characterisation

The Cape Verde warbler has a complex song and call repertoire and does not seem to combine syllables, elements or phrases in long continuous “song bouts”, separated by pauses of relatively constant length, as in many species (e.g. Mortega *et al.* 2014; Palmero *et al.* 2012). Instead, it utters, and often repeats, short combinations of different syllables, elements, or motifs with pauses of unpredictable length between them. Each individual can sing several song types, and no one song type can be classified as the commonest within a population (see supplementary material). Conversely, the type of elements and their combinations seem relatively consistent within a population, suggesting moderate differentiation (Table 3.1 and Figure 3.4). For example, on Santiago individuals seem to repeat a certain element or motif a random number of times, and then sing another motif, or a variation of the same one. In contrast, on Fogo most songs seem to consist of a long repetition of short and monotonous elements, which may be preceded or not by a different element and repeated or not, with marked difference in individual element use. On S. Nicolau, the birds seem either to repeat complex elements or to combine different polytonic elements with trills and whistles or more elements.

Table 3.1 Number of song types, and maximum variations within song type, per individual bird in each population of the Cape Verde warbler. I show the average and standard error (SE) and the maximum and minimum number of maximum song types and variations found within that island.

	Song types per individual				**Variations within song type**				
Island	**Average**	**SE**	**Maximum**	**Minimum**	**Average**	**SE**	**Maximum**	**Minimum**	**N**
Santiago	2.90	0.53	7	1	2.90	0.72	9	1	10
Fogo	2.80	0.51	5	1	2.90	0.41	5	1	10
S. Nicolau	2.67	0.37	4	1	3.22	0.46	6	2	9
Average	2.79	0.27	5.33	1	3.00	0.31	6.67	1.33	29

Individual male Cape Verde warblers can sing at different rates, with pauses between songs that can range from 4 to >30 seconds and song rates between 2.4 and 11.6 songs per minute (2014 recording; data not shown). Individuals either repeat the same song type, switch song types or alternate repetitions with switching (data not shown). The songs are often, but not always, preceded by calls, and sometimes individuals utter calls in between consecutive songs (see supplementary material). In general, the birds utter one or two calls before each song, and calls can be composed of one or two elements, in ascending or descending frequencies.

Frequencies range between a lowest frequency (5% frequency) of 1033.6 Hz and a highest frequency (95% frequency) of 4823.4 Hz (Table S3.7). Length of song (delta time) varies between 0.118 and 2.691 seconds (Table S3.7). The lengths of the pauses between songs and calls, or between songs, or between calls, are variable: the pause between a call and the following strophe can last from 0.15 to 0.70 seconds (data not shown). There are elements shared among individuals and certain elements are common to Santiago, Fogo and S. Nicolau (data not shown); however, the small sample size is too small to confirm I have sampled most of the repertoire on each island and assess which songs are unique to each island (Mortega *et al.* 2014). The different ways in which the warblers can combine and modulate song elements, combined with the limited sample size, make it extremely difficult to characterise and quantify a “typical” song.

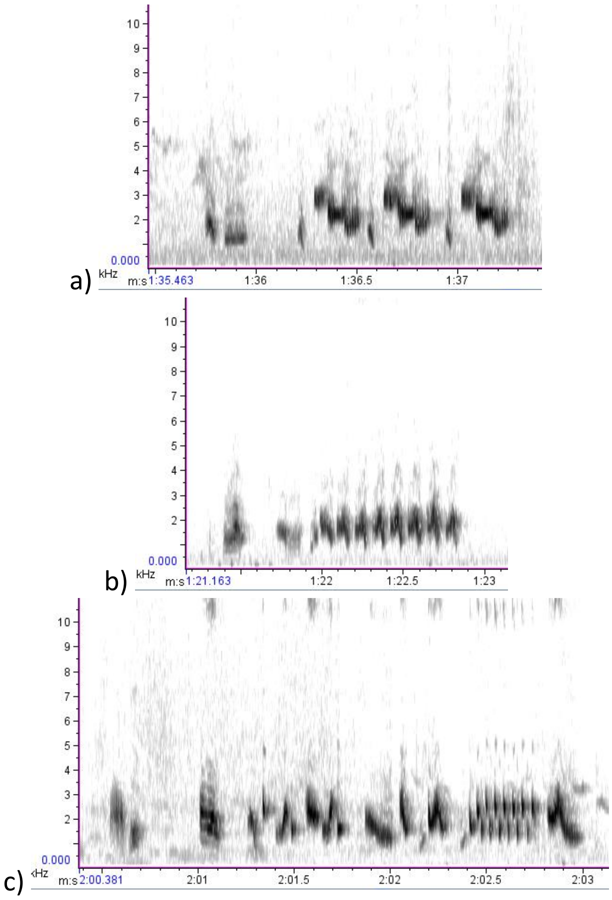


Figure 1.4 Examples of song types in the Cape Verde warbler from each of the three island populations: a) Santiago, a) Fogo and c) S. Nicolau. On Santiago there are usually few repetitions of one syllable, on Fogo few repetitions of initial syllables followed by many repetitions of another syllable, and on S. Nicolau a large combination of different syllables, with few repetitions and often trills. Note that here I show only one song type per island, but each male sings several song types that may or may not be similar (Tables 3.1 and S3.1). *Figures produced using original data on Raven Pro 1.4 by Helena Batalha.*

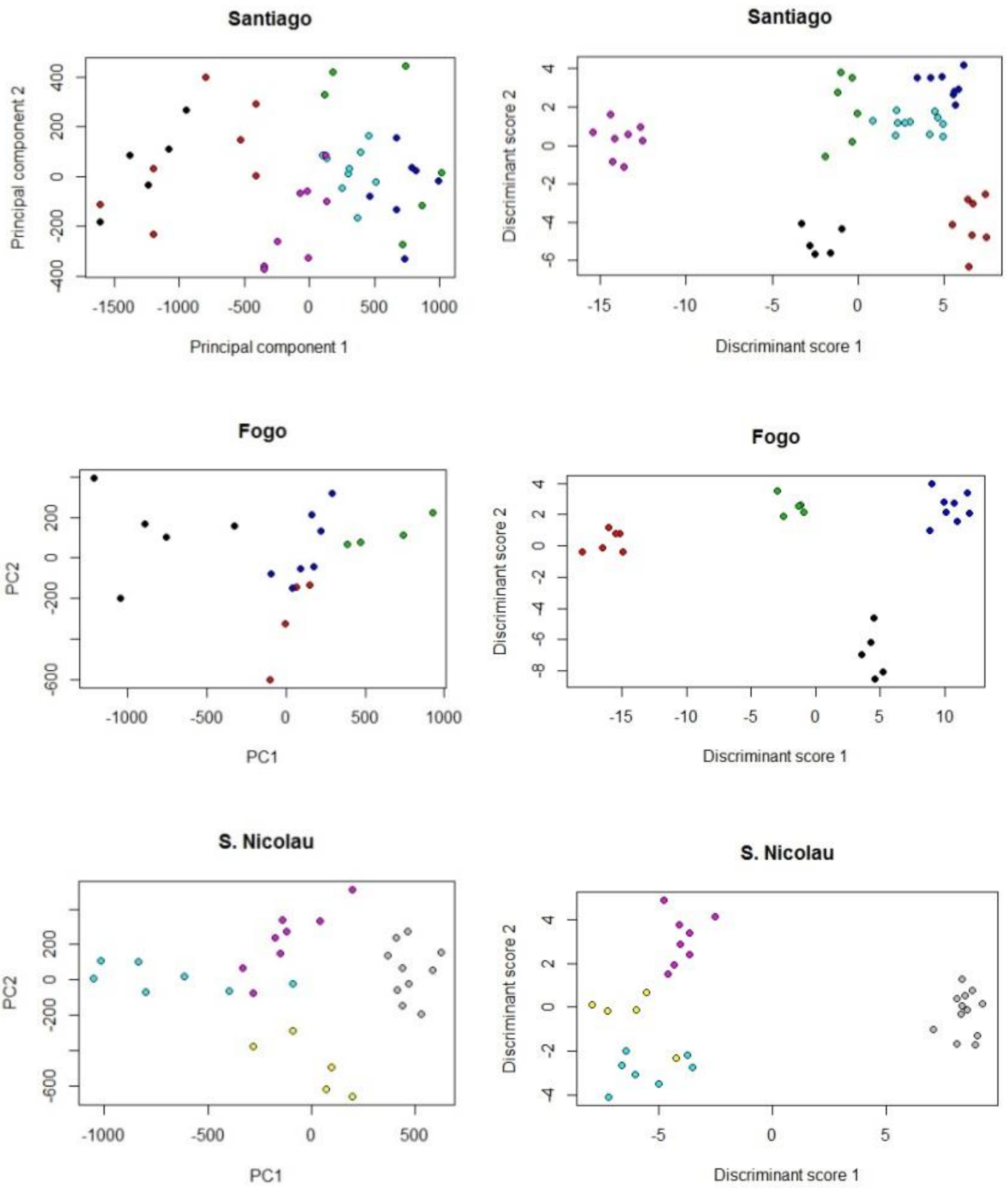
The first principal component (PC1) of the PCAs on spectrogram song types explained more than 70% of the variance in each population: 86.12% on Santiago, 79.00% on Fogo and 71.63% on S. Nicolau (Table 3.2). PC1 was significantly different between spectrogram types in all populations (Table 3.2, Figure 3.5). The second principal components (PC2) explained just between 6.39% and 21.60% of the variance in spectrogram characteristics (Table 3.2). The main loadings in the first PC were the highest frequency values (95% and Q3 frequencies; see Methods for definitions and supplementary material). Some VIF values were above the cut-off value of 10, which indicates multicollinearity between independent variables, so I removed redundant variables from the LDA (aggregated entropy and Q1 and Q3 frequency). Wilks' lambda was extremely low in all populations (Table 3.3), indicating that the within-group (i.e. within-song type) variance is negligible when compared to variance between groups (Figure 3.5). The first discriminant functions explained more than 80% of the variance between spectrogram types in all populations, while the second discriminant function explained less than 15% (see supplementary material). The main loadings in the discriminant functions were the duration of the spectrogram (delta time) and entropy measurements (see supplementary material). Taken together, these results indicate that the largest part of the variance in individual spectrogram measurements is explained by changes in the highest frequencies at which the birds sing, and the largest part of the variance between song types is explained by how long the songs last and their entropy, i.e. if they are more monotonous or more complex in frequency use. They also indicate that energy-based spectrogram measurements can be used to discriminate song types reliably. In other words, if song types on different populations have different traits, measuring spectrograms will be sufficient to discriminate between populations.

Table 3.2 Principal components analysis on different song types on each population of the Cape Verde warbler. From the PCA I report eigenvalues and percentage variance in spectrogram characteristics explained by the first two principal components (PC1 and PC2); from the ANOVA on PC1 I report the F statistic, the degrees of freedom and the p value.

		eigenvalues		**% variance**		**ANOVA (PC1)**		
Population	**n**	**PC1**	**PC2**	**PC1**	**PC2**	**F-statistic**	**df**	**p-value**
Santiago	46	514400	38142.48	86.12	6.39	58.82	5	< 0.001
Fogo	24	263858.4	45048.91	79	13.49	46.15	3	< 0.001
S. Nicolau	32	230110.4	69398.97	71.63	21.6	50.75	3	< 0.001

Table 3.3 Discriminant analysis on different song types on each population of the Cape Verde warbler. I report Wilks' lambda (MANOVA) on discriminant functions between song types on each population, the chi-square test value, the degrees of freedom and the p value.

Population	Wilks' Lambda	Chi^2-Value	DF	p-value
Santiago	0.00028	322.7	25	< 0.001
Fogo	0.00018	159.38	15	< 0.001
S. Nicolau	0.00117	178.83	15	< 0.001



a)

b)

c)

Figure 3.5 Principal components analysis (left) and discriminant analysis (right) on song types for each population of the Cape Verde warbler: a) Santiago, n = 46, b) Fogo, n = 24 and c) S. Nicolau, n = 32. Analyses were made with traits measured from spectrograms; within each plot, each colour codes for a different song type. See supplementary materials for more details.

Song differences between populations

The first PC explained 76.59% of the variance in traits of spontaneous songs during the breeding season and the second PC explained 17.19%. Eigenvalues are 546884.30 for PC1 and 122747.70 for PC2. The ANOVA of PC1 showed significant differences between both islands and individuals (Table 3.4). However, the plotted PCA scores clustered together whether they were grouped by population or by bird (Figure 3.6). All VIF values were below the cut-off value of 10, but I removed the same redundant variables as above to avoid unnecessary multicollinearity (Table S3.2). Wilks' lambda was large for islands and small for individual birds (Table 3.4), indicating that within-island variance is large when compared to between-island variance, and that the opposite is true for individuals. Taken together, these results indicate that spectrogram traits are different between both birds and islands, but while the differences between birds are relatively large, the differences between islands are quite small. In other words, variance is larger between individuals than between islands. The first discriminant function explained 54.47% of variance between islands, and the second discriminant function explained 45.53%. In this case, since there are two discriminant functions and three islands, the first discriminant function separated Santiago from the other two islands, and the second separated Fogo from S. Nicolau (black, red and green circles in Figure 3.6, respectively). However, the plotted LDA scores of different groups all clustered together, especially between islands (Figure 3.6).

Table 3.4 Principal components analysis and discriminant analysis on songs of each population of the Cape Verde warbler, n = 266. From the PCA I report ANOVA on the first principal component (PC1). From the discriminant analysis I report Wilks' lambda (MANOVA) on discriminant functions between populations (first row) and individuals (second row).

	ANOVA (PC1)			**Wilks' lambda (MANOVA)**			
	Df	**F-statistic**	**p-value**	**Wilks' lambda**	**Df**	**Chi^2**	**p-value**
Island	1	7.43	<0.001	0.67	10	104.80	<0.001
Bird	14	16.95	<0.001	0.04	80	823.14	<0.001

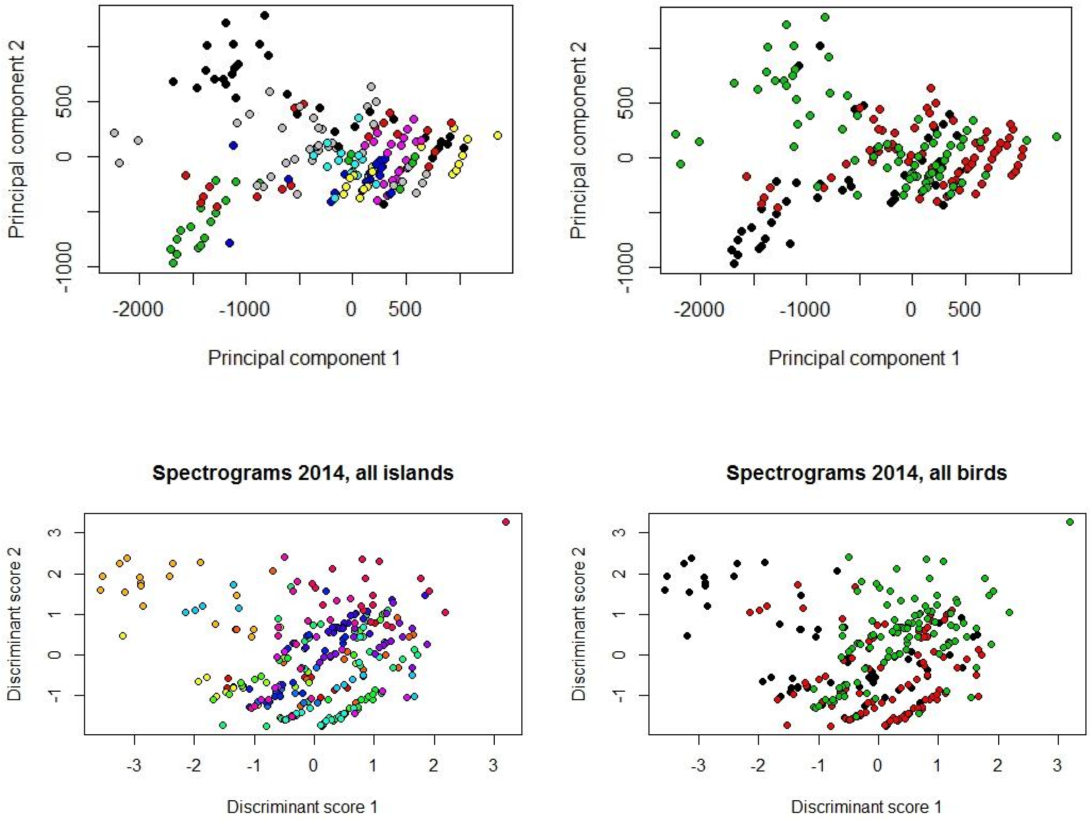


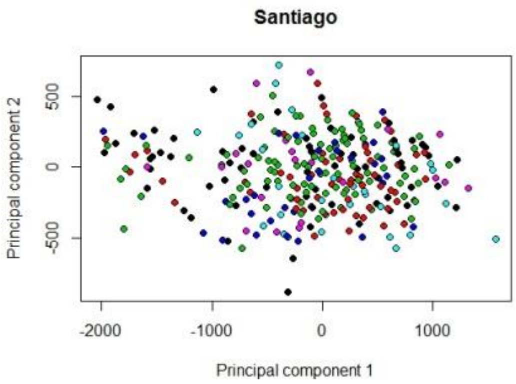
Figure 3.6 Principal components analysis (upper row) and discriminant analysis (lower row) on songs of all populations of the Cape Verde warbler, n = 266. Individual spectrograms coloured by bird (left) and island (right): spectrograms of birds of Santiago in black, Fogo in red and S. Nicolau in green.

Differences in song response to playback

I conducted 36 playback tests, 12 on each island (Figure S3.4). The first PC explained 81.09% of the variance in spectrogram measurements for Santiago, 78.10% for Fogo and 77.82% for S. Nicolau. Eigenvalues for PC1 were 451992.30 for Santiago, 387514.00 for Fogo and 358074.70 for S. Nicolau. Spectrograms clustered together within each island, not clustering separately according to which song the birds were responding to (Figure 3.7). I tested differences in songs in response to different populations of origin using just PC1 (Table 3.5). There are no differences in the spectrograms of the songs used to respond to playbacks from six different populations of origin, on either island (all $p > 0.130$; Table 3.5). However, trial order seems to have a significant negative effect on the island of Fogo ($p = 0.003$; Table 3.5). The PC1 on Fogo mostly reflects the highest frequencies in each song (negative loadings of 95% and Q3 frequency measurements; see supplementary table S3.8), and trial order is ranked from one to six (i.e. from the initial LSW, used to detect the birds, to the last song used in the playback test).

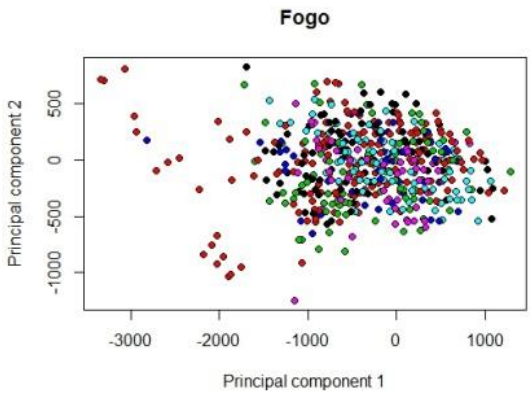
Table 3.5 Linear mixed-effects model for song response to playback tests on Santiago, Fogo and S. Nicolau. Estimates represent differences in PC1 of the spectrogram traits of songs uttered in response to songs of each of the populations. Intercept represents the population of origin. Birds were included as random effects. Significant effects are shown in bold.

		Intercept	**Santiago**	**Fogo**	**S. Nicolau**	**GSW**	**SW**	**LSW**	**Trial order**
Santiago	Estimate	-38.260		101.850	19.110	49.790	115.420	264.120	41.050
	SE	186.900		102.080	102.930	116.990	118.940	170.000	25.520
	df	40.120		28.410	28.510	33.710	46.320	32.170	34.940
	t value	-0.205		0.998	0.186	0.426	0.970	1.554	1.609
	P value	0.839		0.327	0.854	0.673	0.337	0.130	0.117
Fogo	Estimate	194.940	-157.240		19.020	87.240	58.160	-163.400	**-63.500**
	SE	155.480	132.700		136.090	135.050	133.300	173.080	**21.370**
	df	53.890	39.090		41.580	41.220	44.790	56.090	**211.450**
	t value	1.254	-1.185		0.140	0.646	0.436	-0.944	**-2.972**
	P value	0.215	0.243		0.890	0.522	0.665	0.349	**0.003**
S. Nicolau	Estimate	36.524	-116.039	-134.646		-237.654	-13.176	-67.535	5.417
	SE	198.225	163.466	143.944		155.223	143.802	217.710	39.960
	df	42.630	32.290	30.690		30.210	30.900	38.690	39.170
	t value	0.184	-0.710	-0.935		-1.531	-0.092	-0.310	0.136
	P value	0.855	0.483	0.357		0.136	0.928	0.758	0.893

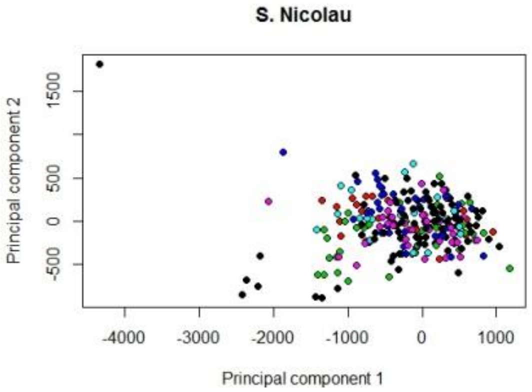


a)

Figure 3.7 Principal components analysis of variance in song uttered in response to different populations of origin in the Cape Verde warbler. Songs coloured by origin of the song the birds were responding to; a) Santiago, n = 266, b) Fogo n = 1425 and c) S. Nicolau, n = 451.



b)



c)

Figure 3.7 (Cont.)

Differences in behavioural response to playback

Latency to approach and time spent within 5 m were strongly negatively correlated, and number of calls and songs were strongly positively correlated (Spearman's r2 = 0.95 and 0.75, respectively). Thus I retained only latency to approach, minimum distance to speaker and number of songs in the following analyses. ANOVAs and AIC selected the full model for latency to approach on Fogo and S. Nicolau and minimum distance to speaker on Fogo and the reduced model for all the other tests (Table S3.9). Since the main objective of these models was not to explain variance in behavioural measurements but to test for differences in warbler reactions to songs of different populations, I kept all the variables of interest in the models. Overall there were no significant differences between the behavioural responses of birds to songs of different populations/species (Tables 3.6 to 3.8). Trial order had a positive effect on the latency to approach for birds of Fogo and S. Nicolau (Table 3.6) and a negative effect on minimum distance to speaker for birds of Fogo (Table 3.7).

Table 3.6 Cox survival mixed model on latency to approach within 5 m for playback tests on Santiago, Fogo and S. Nicolau. Estimates represent differences from the latency to approach to songs of the population of origin (intercept, not shown); n= 12. Significant differences are shown in bold. See supplementary material for more details.

		Santiago	**Fogo**	**S. Nicolau**	**GSW**	**SW**	**Trial order**	**Song rate**
Santiago	coef		0.067	0.638	-1.048	-1.442	0.168	0.030
	exp(coef)		1.069	1.893	0.351	0.237	1.183	1.030
	se(coef)		0.534	0.583	0.601	0.751	0.152	0.093
	Z value		0.130	1.090	-1.740	-1.920	1.110	0.320
	P value		0.900	0.270	0.081	0.055	0.270	0.750
Fogo	coef	0.136		-0.293	-0.370	-1.151	**0.376**	0.187
	exp(coef)	1.146		0.746	0.691	0.316	**1.457**	1.205
	se(coef)	0.571		0.582	0.604	0.665	**0.122**	0.108
	Z value	0.240		-0.500	-0.610	-1.730	**3.090**	1.740
	P value	0.810		0.610	0.540	0.084	**0.002**	0.082
S. Nicolau	coef	-0.986	-0.704		-0.974	-0.253	**0.419**	-0.042
	exp(coef)	0.373	0.495		0.378	0.776	**1.521**	0.959
	se(coef)	0.561	0.529		0.617	0.584	**0.142**	0.088
	Z value	-1.760	-1.330		-1.580	-0.430	**2.960**	-0.470
	P value	0.079	0.180		0.110	0.660	**0.003**	0.640

Table 3.7 Linear mixed model on minimum distance to speaker for playback tests on Santiago, Fogo and S. Nicolau. Estimates represent differences from the minimum distance to speaker with songs of the population of origin (intercept); n= 12. Significant differences are shown in bold. See supplementary material for more details.

		Intercept (own population)	**Santiago**	**Fogo**	**S. Nicolau**	**GSW**	**SW**	**Trial order**	**Song rate**
Santiago	Estimate	3.332		1.446	0.100	3.646	2.821	0.695	-0.241
	Std. Error	3.171		2.164	2.148	2.158	2.504	0.513	0.365
	df	54.310		44.350	43.590	43.910	46.430	45.160	48.480
	t value	1.051		0.668	0.046	1.690	1.127	1.356	-0.662
	P value	0.298		0.508	0.963	0.098	0.266	0.182	0.511
Fogo	Estimate	**10.271**	-2.816		0.054	0.197	-0.276	**-1.399**	-0.162
	Std. Error	**3.281**	2.106		2.149	2.122	2.388	**0.464**	0.344
	df	**56.970**	46.230		45.680	45.540	47.010	**45.480**	51.170
	t value	**3.130**	-1.337		0.025	0.093	-0.116	**-3.014**	-0.470
	P value	**0.003**	0.188		0.980	0.927	0.908	**0.004**	0.640
S. Nicolau	Estimate	-0.642	3.854	4.538		5.146	-1.278	-0.372	0.722
	Std. Error	4.451	3.010	3.043		3.508	3.584	0.762	0.506
	df	46.990	36.920	37.250		38.620	40.530	37.270	46.940
	t value	-0.144	1.280	1.491		1.467	-0.356	-0.488	1.426
	P value	0.886	0.208	0.144		0.151	0.723	0.628	0.161

Table 3.8 Linear mixed model on number of songs for playback tests on Santiago, Fogo and S. Nicolau. Estimates represent differences from number of songs in response to songs of the population of origin (intercept); n= 12. See supplementary material for more details.

		Intercept (own population)	**Santiago**	**Fogo**	**S. Nicolau**	**GSW**	**SW**	**Trial order**	**Song rate**
Santiago	Estimate	2.944		0.754	3.287	-0.441	0.592	0.234	-0.162
	Std. Error	1.693		1.259	1.277	1.259	1.411	0.291	0.197
	df	58.680		48.010	48.090	48.030	50.140	48.220	57.110
	t value	1.739		0.599	2.574	-0.350	0.420	0.803	-0.822
	P value	0.087		0.552	0.013	0.728	0.676	0.426	0.415
Fogo	Estimate	3.763	0.980		1.494	-0.540	-2.391	0.278	0.350
	Std. Error	2.411	1.328		1.334	1.317	1.555	0.300	0.224
	df	48.490	48.000		48.060	48.010	48.560	48.000	49.930
	t value	1.561	0.738		1.119	-0.410	-1.538	0.927	1.563
	P value	0.125	0.464		0.269	0.683	0.131	0.358	0.124
S. Nicolau	Estimate	2.322	-1.394	-1.245		-0.603	-1.336	0.401	0.195
	Std. Error	1.953	1.318	1.327		1.395	1.485	0.312	0.204
	df	59.210	48.040	48.100		48.200	49.470	48.000	54.240
	t value	1.189	-1.057	-0.939		-0.432	-0.900	1.284	0.954
	P value	0.239	0.296	0.353		0.668	0.373	0.205	0.344

3.5 Discussion

In this study, I found no evidence for song divergence between the three populations of the Cape Verde warbler. I also found no evidence that the males of any population respond differently, in song or behaviour, to songs of other populations of origin, or even of other *Acrocephalus* species. Collectively, my results suggest that song plays a very limited, or no role in driving divergence or reproductive isolation between populations in this species. However, male reaction to playbacks does not always correspond to female preferences (Seddon & Tobias 2010; Tobias & Seddon 2009), thus inferences about the role of song in reproductive isolation in the Cape Verde warbler are speculative at this point.

Several processes can drive song divergence in different populations of oscine passerines over relatively short periods of evolutionary time. For example, cultural drift can lead to song diversification through the accumulation of copying errors (Nottebohm 1968, 1969). This process can occur rapidly and lead to strong divergence in very short evolutionary timescales (Marler & Slabbekoorn 2004; Slabbekoorn & Smith 2002), e.g. 50 years (Baker *et al.* 2003). As the three Cape Verde warbler populations have been separated for 165–199 Kya, the stochastic accumulation of differences between populations was a plausible possibility, but my results do not support this hypothesis. Natural, sexual and cultural selection on bird song can also lead to the evolution of differences between populations (reviewed in Podos *et al.* 2004; Price 1998). However, I found no evidence for selection in song acting on any of the populations.

3.5.1 Cape Verde warbler song

The song of the Cape Verde warbler is composed of small motifs arranged in various combinations, interspersed with a variety of calls and pauses, as seen in other *Acrocephalus* warblers (Leisler & Schulze-Hagen 2011). Each male sings a variety of song types and respective variations, and while there are apparent population-specific trends, i.e. more complex arrangements on S. Nicolau, there are some motifs and arrangements in common between all the populations. Overall, Cape Verde warbler songs have a low frequency (ca. 1000–5000 Hz, Table S3.7), lower than those of some congeneric Palearctic species, such as the marsh warbler *A. palustris* (Dowsett-Lemaire 1979). Low-frequency songs are favoured in densely vegetated habitats where they are transmitted better than higher-frequency songs (Derryberry 2009; Morton 1975; Slabbekoorn & Smith 2002). Cape Verde warblers live in densely vegetated habitat (Batalha *et al.* 2017; Garcia-del-Rey 2016), and habitat characteristics might favour low-frequency songs and calls in this species (Giuseppe & Saino 2007). Furthermore, densely vegetated areas are limited in the Cape Verde archipelago, which has a year-round dry climate interrupted by a brief, unpredictable rainy season (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986). Thus, competition for space could increase the intensity of selection for low-frequency and varied song types, and aggressive signalling.

There are apparent but ambiguous differences between the spontaneous songs of the three Cape Verde warbler populations (Table 3.3, Figure 3.4). Songs of the Santiago population seem more diverse, both within and between individuals, than those of Fogo and S. Nicolau (Table 3.1). The song diversity pattern across populations partially matches the patterns of genetic diversity observed in this warbler: Santiago is the largest, most widespread and most genetically diverse population, while S. Nicolau is the least diverse, smallest and most geographically concentrated (Batalha *et al.* 2017). Song diversity in Santiago thus matches the relatively high genetic diversity in this population, while the reduced song diversity in S. Nicolau could be a result of cultural or genetic drift following a population bottleneck (Batalha *et al.* 2017). However, Fogo does not seem to fit in this pattern, because its song diversity seems to be as low as the one of S. Nicolau (Table 3.1). This could be due to cultural drift in a dense and geographically localised population, where any copying errors could be easily transmitted to all individuals, or to selection for particular song types occurring in Fogo (Podos *et al.* 2004).

Discrimination between different song types in the Cape Verde warbler was possible using energy-based measurements on spectrograms (Table 3.1). The measurements that had most variance were the highest frequencies of songs (95% frequencies). This could be because vegetation density at the locations where birds were recorded obstructed transmission of different frequencies or, alternatively, because there is variation in the extent to which birds include high-frequency elements in their songs. The measurements that most differed between song types were their duration and their entropy. Song duration differed between song types because of the number of syllables and repetitions included in each type. Entropy measurements, which have been successfully used to characterise animal sounds in recent studies (Fernández-Vargas & Johnston 2015; Schwabl *et al.* 2015), reflect the purity of the tones used by the animals, or the variation in frequencies used in each song. In other words, lower values of average entropy indicate purer tones and higher values are characteristic of tones that cover a larger bandwidth; lower values of aggregated entropy indicate more monotonic songs and higher values characterise songs with more variation in their inclusion of high- and low-frequency elements. Taken together, my results demonstrate that, in the Cape Verde warbler, different song types are characterised by different numbers of elements and their repetitions, and also by different degrees of inclusion of high and low frequency elements.

My results contrast with two general trends in bird song. Bird song complexity increases with latitude (Kaluthota *et al.* 2016); thus species living close to the Equator should sing simpler songs. It has also been shown that island bird song tends to be less complex when compared to mainland species (Morinay *et al.* 2013). The fact that there is large intra- and inter-individual variability in the Cape Verde warbler's song suggests it may not conform to either of these general patterns, but I did not examine variables related to song complexity. As most studies on avian song have been conducted in temperate zones, comparatively little is known about song in species that live in harsher, species-poor environments (Marler & Slabbekoorn 2004; Singh & Price 2015). It has been suggested that songs in *Phylloscopus* warblers have evolved to be complex in species-poor and relatively quiet environments (Singh & Price 2015), and the same could have happened in the Cape Verde warbler, which inhabits a similar environment (pers. obs.). Other findings suggest that birds subjected to variable and unpredictable climates display more complex songs (Botero *et al.* 2009). A hypothesis recently proposed to explain this pattern is that such climates could drive evolution of signals of intelligence in birds, and this translates as the capacity to improvise songs (Botero *et al.* 2009; Catchpole 1996). Harsh climates such as on Cape Verde could have exerted strong selective pressures in the warblers.

3.5.2 Responses to playback

Cape Verde warbler males do not appear to discriminate between males of their own versus other conspecific populations, or even versus males of different species. This contrasts with the behaviour of species which respond more aggressively to conspecifics than heterospecifics (Grant & Grant 2002; Mortega *et al.* 2014). However, it is unclear if this is the result of real lack of discrimination by the Cape Verde warblers or if it is an artefact caused by large intra- and inter-individual song diversity. Small intra-population and/or large inter-individual differences in response to songs, associated with a small sample size, could explain the large effect sizes and the non-significance of variables observed in playback test models (Tables 3.5 to 3.8). There is large intra-individual variation in the way that Cape Verde warblers sing, which can confound the behavioural and song responses they give when confronted with “intruders” of different origins (Fisher 1954), and the same holds true for their behavioural responses (latency to approach, minimum distance and number of songs). In many instances, individual birds reacted very similarly to all five different songs that they were played, suggesting that response intensity is not linked to characteristics of the individual song that was used in playback trials, as in Peake *et al.* (2002), but to the those of the responding bird. It is also possible the warblers are not accustomed to having “intruders” and react to any novel bird that comes into their territory. For example, some birds react aggressively to potential predators or parasitic bird species, not just to potential competitors (e.g. Neudorf & Sealy 1992).

The methods I used to locate Cape Verde warbler territories were chosen to maximise the number of data points obtained in the time I had available. After confirming that the warblers do not inhabit sparsely vegetated or barren areas, I based my surveys in a combination of previously recorded information, recent observations from local biologists and my own assessment of areas occupied by the warbler from previous fieldwork (Batalha *et al.* 2017). Within the broad category of “densely vegetated” areas occupied by the warbler, I aimed to survey all possible habitat types (sugarcane plantations, mountain forests, reed stands, etc.) and all vegetated altitudes (from sea level to mountain tops) on all areas of each island. Because I actively targeted locations where I thought the warbler lived, the test points are not completely randomly distributed, and the probability of finding the birds is artificially high. However, because they were still spread across various habitats, altitudes and geographic areas, it is unlikely that this had an effect in the outcome of the tests.

A possible explanation as to why Cape Verde warblers did not react differently to songs of the greater swamp warbler concern whether they are effectively reproductively isolated. Genetic divergence between the two sister species is limited, with an estimated date of divergence only within the last half-million years (Batalha *et al.* 2017). They have also been described as having similar colour, vocalisations and breeding behaviour (Hazevoet 1995). It is not inconceivable that the speciation process between these two taxa is still incomplete and the boundaries between the two are not yet consolidated by full reproductive isolation. Territorial responses of males to songs of males of other populations are often used to assess the degree of reproductive isolation between two populations, as a proxy of female reactions (Balakrishnan & Sorenson 2006; Grant & Grant 2002; Irwin *et al.* 2001). However, in playback tests conducted in antbirds, *Hypocnemis peruviana* females were able to discriminate between conspecifics and heterospecifics, even though males reacted aggressively to conspecific and heterospecific songs in sympatric areas (Seddon & Tobias 2010; Tobias & Seddon 2009). Thus a lack of discrimination by males, and even similarity between mating signals, does not necessarily imply a lack of discrimination by females. While the behavioural responses of the Cape Verde warbler males suggest more fluid reproductive boundaries, this would need to be confirmed with behavioural tests on females or percentage divergence on DNA using a large enough sample size. In the *Hypocnemis* antbirds tested divergence between species tested was 6.8% in mitochondrial DNA (Seddon & Tobias 2010; Tobias *et al.* 2008). Currently, divergence between the greater swamp warbler and the three populations of the Cape Verde warbler is estimated to range from 0.4 - 0.5% for the cytochrome b gene (Batalha *et al.* 2017), but since only seven greater swamp warbler samples were used in this analysis results are not conclusive.

Bird songs are often used for intrasexual competition and territoriality while bird calls are less strongly related to territoriality and reproduction (Catchpole 1983; Catchpole & Slater 2008). Standard playback tests use bird song only (Grant & Grant 2002; Mortega *et al.* 2014; Seddon

& Tobias 2010), thus clearly evaluating individual responses to playbacks in a context of sexual selection. Song in the Cape Verde warbler is currently very poorly studied (Cramp & Perrins 1992; Donald *et al.* 2004; Hazevoet 1995) as are the relative roles of songs and calls in mating attraction and competition during the breeding season. Because of this, and because Cape Verde warblers naturally often intermix calls with songs, I chose to integrate calls in the playback recordings used in the tests. However, this might have had an influence in the outcome of the playback tests, i.e. responding males may have perceived the playbacks more as competition for space and food than for females (Seddon & Tobias 2010).

In the song responses to playback tests on the Fogo population, the highest frequencies of the response songs increase as the trial order increases (Table 3.5). Low song frequencies have been linked to aggressiveness in birds (Benedict *et al.* 2012; Morton 1977), and it is possible that Fogo males react aggressively to the first intruders, uttering lower frequency songs, and gradually lose interest, consequently increasing the frequency of their response songs. Accordingly, there was a positive effect of trial order on latency to approach on Fogo and S. Nicolau. In other words, the more test songs are played, the longer it takes for the tested bird to come within five meters of the speaker. Again, this could happen simply because the birds start to lose interest in the “intruder”, although it is unclear why that happens in such small, localised populations and not on the widespread population of Santiago. Trial order had a negative effect on the minimum distance to speaker on Fogo, which means that the more songs are played back, the closer the tested bird approaches the speaker. This could be because the birds start by carefully attempting to assess the “intruders” and evaluating the potential risks of starting a fight, and as they lose fear and/or aggressiveness, they start approaching the “intruders”. However, as mentioned before, low sample size and high variability in responses mean that any inferences must be taken with caution.

Species with large repertoires are less suitable for playback tests than those with short repertoires because they do not respond consistently to aural challenges (Boughey & Thompson 1976). The degree of variation in motif arrangement in Cape Verde warbler songs is reminiscent of birds such as the brown thrasher *Toxostoma rufum*, the marsh warbler, the spectacled warbler *Sylvia conspicillata* and the red-faced cisticola *Cisticola erythrops*, i.e. species with large repertoires and inter-individual variability in song (Benedict & Bowie 2009; Boughey & Thompson 1981; Dowsett-Lemaire 1979; Palmero *et al.* 2012). Playback experiments produce clearer results with species with a more stereotyped song such as the stonechat *Saxicola torquata*, Darwin's finches *Geospiza* spp., song sparrow *Melospiza melodia* and even other *Acrocephalus* warblers (Catchpole & Leisler 1986; Grant & Grant 2002; Mortega *et al.* 2014; Searcy *et al.* 1997). In addition to the intrinsic complexity of the warbler's song, time constraints and the limited number of territories on S. Nicolau (Batalha *et al.* 2017) means that only 12 playback tests were conducted on each island. Therefore, all ecological inferences from the results of the playback tests must be interpreted with caution.

Several hypotheses could explain intra- and inter- individual differences in song and response to playback. One of the factors I could not control for was the age of individual males because I did not know in which year they were born. It is possible that older males react more aggressively to intruders' songs (whether intruders come from their own population or not) or are generally more aggressive (Hyman *et al.* 2004). If the warblers incorporate elements of their neighbours' and other species' vocalisations in their repertoire, as seen on the marsh warbler (Dowsett-Lemaire 1979), it is also possible that older males have a larger repertoire and thus appear to display more variation in song. However, there are no studies on the avian community composition on each of the islands and areas inhabited by the Cape Verde warbler to confirm or discard this hypothesis. Alternatively, it might be possible that conducting the playback tests outside key periods of the breeding cycle caused male response to be inconsistent. Another hypothesis is that the warblers might actually display behavioural syndromes/personalities (Dingemanse *et al.* 2010; Jacobs *et al.* 2014; Sih *et al.* 2004). Overall it seems that the song challenges elicited an inconsistent response from individuals from the three warbler populations, suggesting that either behavioural processes mask a potential song differentiation, or that song in this species is not, or only weakly, under selection.

Conclusions

This study demonstrates that the level of acoustic variability is high in the Cape Verde warbler, and reveals no divergence in song, or response to song, between the three populations of Cape Verde warbler. Therefore, I found no support for the hypothesis that song may play a role in driving or reinforcing divergence or reproductive isolation between populations in this species.

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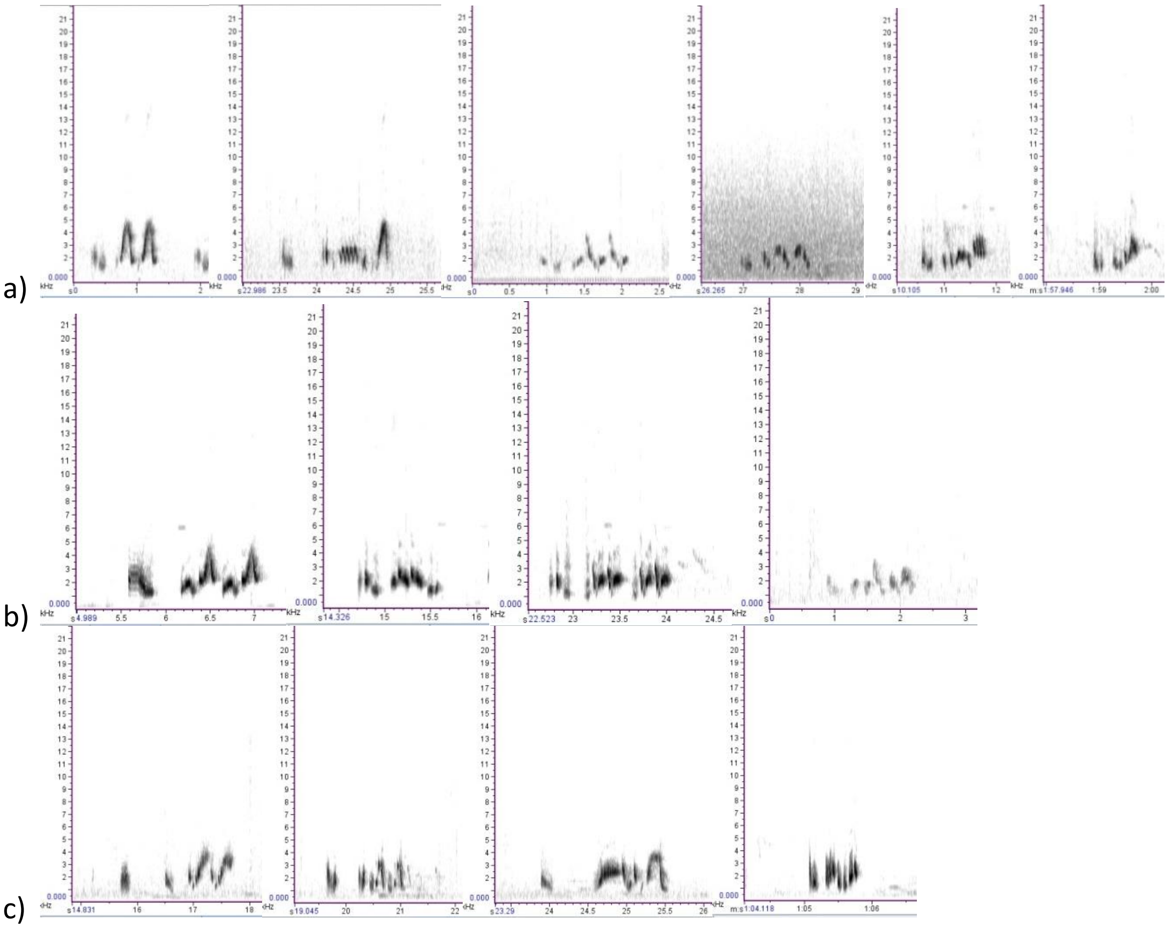
Supplementary table S3.1 Repertoire size per male for the three populations of the Cape Verde warbler. Number of songs, song types, and maximum variation within song types for each individual; each male is either coded by colour-ring combination or GPS code of the location where it was recorded (except for one bird on S. Nicolau); songs refer to number of consecutive songs assessed.

Island	Male	Songs	Song types	Maximum variations
Santiago	WX_RB	15	7	2
	WX_WR	11	3	3
	BX_OR	7	2	3
	S01	12	3	3
	233	5	2	2
	230	4	4	1
	212	5	1	2
	195	12	3	1
	223	21	2	9
	215	14	2	3
Fogo	BB_BX	13	5	2
	BB_OX	11	1	4
	BB_RX	18	3	5
	BR_YX	32	5	3
	RO_BX	6	2	1
	RO_RX	7	1	4
	RY_OX	12	1	2
	RY_WX	9	2	2
	151	12	4	2
	AB111	15	4	4
S. Nicolau	RB_XW	18	3	5
	RR_XB	19	1	2
	RR_XO	21	3	6
	RR_XR	15	2	2
	RW_XR	12	4	3
	RW_XW	12	3	3
	RY_XO	10	3	3
	RY_XO	15	4	3
	unringed	5	1	2



Supplementary figure S3.1 Selected song types from the three populations used to test for differences between song types, here shown with the preceding call (not included in the analyses); a) Santiago, b) Fogo, c) S. Nicolau.

Supplementary table S3.2 Variance inflation factors (VIF) for all the variables used in the MANOVAs and linear discriminant analyses (LDA) on characteristics of songs uttered by the Cape Verde warbler spontaneously during the breeding season (2014) and in response to playbacks (2016); the top section shows the initial VIF values, and the bottom section shows the VIF values for the variables retained in the LDA and MANOVA.

			Aggregated entropy	Average entropy	Q1 frequency	Q3 frequency	Center frequency	5% frequency	95% frequency	Delta time
Initial	2014	all	9.20	2.30	5.01	8.11	5.94	3.67	6.80	1.15
	2016	Santiago	9.22	2.23	7.14	9.66	7.95	6.23	10.13	1.85
		Fogo	14.59	9.01	8.93	9.88	5.58	7.54	17.07	8.12
		S. Nicolau	18.97	2.16	5.24	15.66	8.01	6.81	10.02	4.08
Final	2014	all		1.85			2.57	2.04	2.03	1.14
	2016	Santiago		1.81			4.14	2.55	4.10	1.56
		Fogo		6.87			2.63	3.81	2.18	5.58
		S. Nicolau		1.56			5.13	1.73	2.55	3.72

Supplementary table S3.3 Summary statistics of spectrograms traits of songs of the Cape Verde warbler recorded during the breeding season of 2014 (October and November), and used in the discriminant analysis.

		Average entropy	Center frequency	5% frequency	95% frequency	Delta time
All	Min	1.734	1550	1206	1895	0.194
	Median	2.224	2067	1378	2584	1.040
	Mean	2.221	2115	1423	2825	1.147
	Max	3.063	3273	2067	4479	2.600
Santiago	Min	1.813	1723	1206	2240	0.194
	Median	2.212	2240	1550	2756	1.152
	Mean	2.191	2339	1553	2945	1.134
	Max	2.626	3273	1895	4307	2.379
Fogo	Min	1.734	1550	1206	2067	0.548
	Median	2.150	1895	1378	2412	1.020
	Mean	2.144	1978	1388	2570	1.052
	Max	2.572	2756	2067	3962	2.439
S. Nicolau	Min	1.911	1550	1206	1895	0.284
	Median	2.309	2067	1378	2756	1.011
	Mean	2.316	2116	1380	3006	1.251
	Max	3.063	2584	1895	4479	2.600

Supplementary table S3.4 Linear discriminant analysis on song types of the Cape Verde warbler; percentage variance explained (proportion of trace) and coefficients of the linear discriminant functions between spectrogram types; absolute coefficient values > 0.5 are highlighted in bold.

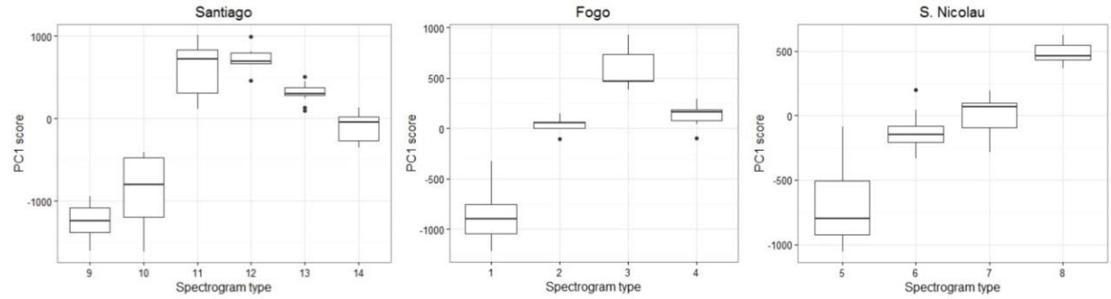
		LD1	LD2	LD3	LD4	LD5
Santiago	Percentage variance	0.815	0.133	0.045	0.008	0.001
	Average entropy	**2.212**	**-0.682**	**5.453**	**5.082**	-0.451
	Center frequency	0.002	-0.002	0.004	-0.006	-0.005
	5% frequency	-0.003	-0.003	-0.009	0.006	-0.004
	95% frequency	0.000	-0.004	-0.001	0.000	0.003
	Delta time	**47.811**	**-1.087**	**-5.039**	**-4.604**	**-2.552**
Fogo	Percentage variance	0.878	0.111	0.011		
	Average entropy	**10.157**	**9.849**	**-7.961**		
	Center frequency	-0.004	-0.002	-0.005		
	5% frequency	0.006	0.004	0.001		
	95% frequency	0.001	-0.008	0.000		
	Delta time	**48.357**	**-6.338**	**10.653**		
S. Nicolau	Percentage variance	0.880	0.087	0.034		
	Average entropy	**1.772**	**7.439**	-0.141		
	Center frequency	0.000	0.000	-0.001		
	5% frequency	-0.003	-0.006	-0.005		
	95% frequency	-0.003	-0.004	0.004		
	Delta time	**-31.072**	**7.078**	**-2.569**		

Supplementary table S3.5 Principal components analysis of song types, on the three populations of the Cape Verde warbler; loadings for the principal components on the three populations; absolute values > 0.5 are highlighted in bold.

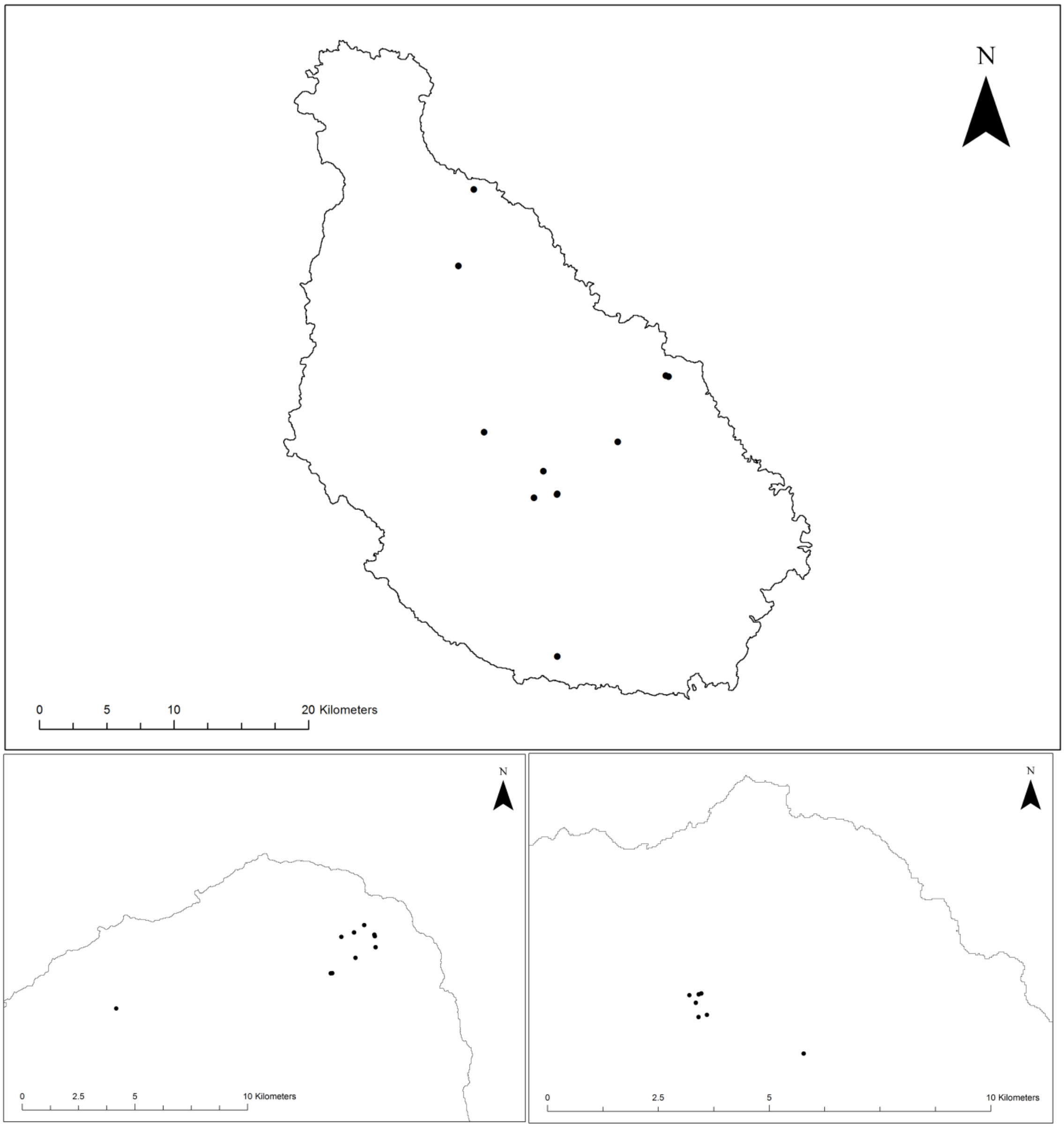
		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Santiago	Aggregated entropy						-0.132	**0.737**	**0.663**
	Average entropy						**0.930**	-0.140	0.340
	Q1 frequency	-0.320	-0.451	-0.483		**0.679**			
	Q3 frequency	-0.493	-0.385	**0.662**	0.413				
	Center frequency	-0.355	-0.392		**-0.683**	-0.498			
	5% frequency	-0.210		**-0.567**	**0.591**	**-0.530**			
	95% frequency	**-0.696**	**0.700**		-0.113	0.105			
	Delta time						0.343	**0.661**	**-0.667**
Fogo	Aggregated entropy						0.299	**0.954**	
	Average entropy						**0.798**	-0.232	**-0.557**
	Q1 frequency	-0.159	**-0.506**			**0.847**			
	Q3 frequency	-0.488		**-0.519**	**0.682**	-0.155			
	Center frequency	-0.271	**-0.548**	-0.373	**-0.606**	-0.346			
	5% frequency	-0.127	**-0.525**	**0.695**	0.309	-0.359			
	95% frequency	**-0.805**	0.405	0.328	-0.266				
	Delta time						**0.524**	-0.190	**0.830**
S. Nicolau	Aggregated entropy						-0.464	**0.687**	**-0.560**
	Average entropy						**0.811**		**-0.580**
	Q1 frequency	-0.217	**-0.565**	-0.276	**0.514**	**-0.542**			
	Q3 frequency	**-0.525**	0.260	-0.444	**-0.567**	-0.372			
	Center frequency	-0.326	-0.196	**-0.533**	0.143	**0.742**			
	5% frequency	-0.163	**-0.723**	0.374	**-0.546**	0.111			
	95% frequency	**-0.737**	0.228	**0.551**	0.310				
	Delta time						0.356	**0.723**	**0.592**

Supplementary table S3.6 Principal component analysis of songs uttered by the Cape Verde warbler spontaneously during the breeding season of 2014, on Santiago, Fogo and S. Nicolau combined; coefficients of the linear discriminants between spectrograms grouped by island; absolute values > 0.5 are highlighted in bold.

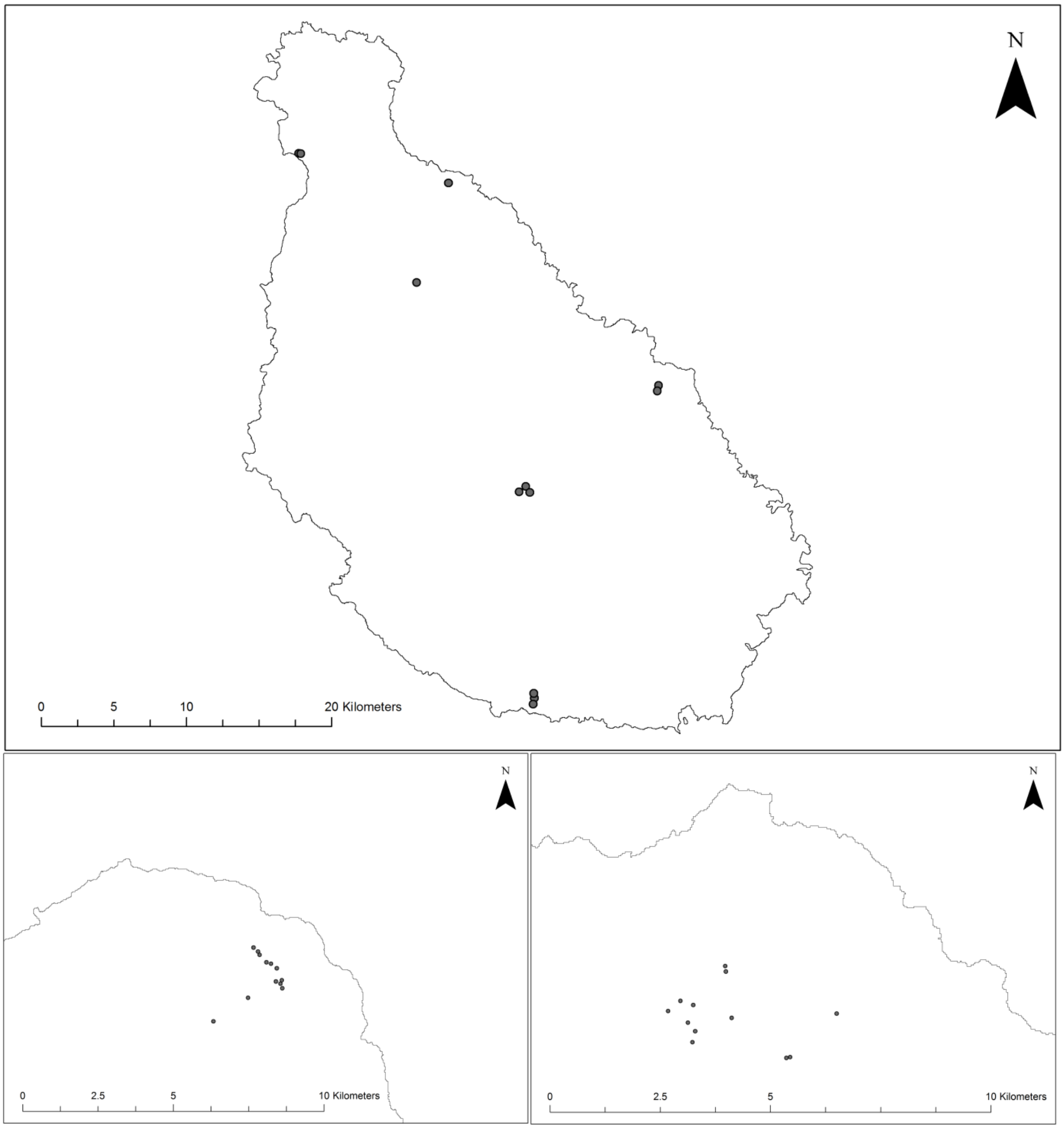
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**LD1**	**LD2**
Aggregated entropy							**0.744**	**0.667**		
Average entropy						0.125	**0.658**	**-0.742**	2.548	1.780
Q1 frequency	-0.201	-0.457	0.383	-0.221	**0.745**					
Q3 frequency	**-0.503**	-0.218	**-0.620**	**0.522**	0.205					
Center frequency	-0.343	**-0.526**	-0.184	**-0.575**	-0.491				-0.003	0.001
5% frequency	-0.132	-0.376	**0.592**	**0.575**	-0.400				-0.002	-0.001
95% frequency	**-0.756**	**0.570**	0.291	-0.129					0.000	0.001
Delta time						**0.991**	-0.117		0.346	0.671



Supplementary figure S3.2 Boxplots of the score of the first principal component of the spectrogram measurements of song types (spectrogram type) uttered by the Cape Verde warblers, on the three existing populations.



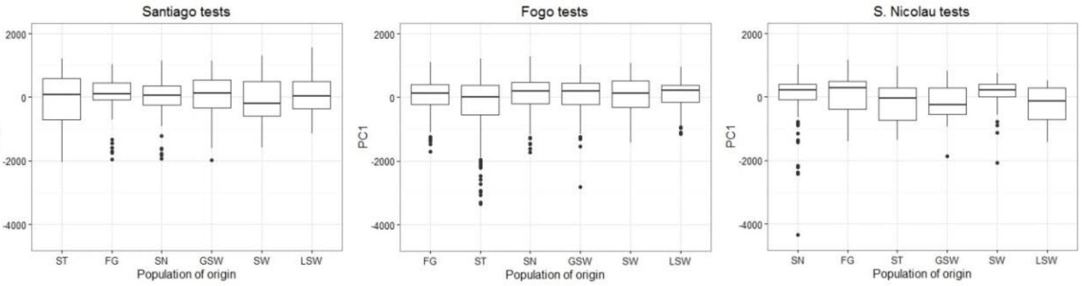
Supplementary figure S3.3 Location of the Cape Verde warbler songs recorded in 2014 and used for the playback tests in 2016: on Santiago (upper panel), Fogo (lower left) and S. Nicolau (lower right). The map scales are 20 km for Santiago and 10 km for Fogo and S. Nicolau.



Supplementary figure S3.4 Location of the playback tests done on Cape Verde warblers in 2016. On each islands, 12 tests were conducted: on Santiago (upper panel), Fogo (lower left) and S. Nicolau (lower right). The map scales are 20 km for Santiago and 10 km for the insets, Fogo and S. Nicolau.

Supplementary table S3.7 Minimum and maximum frequency and length of Cape Verde warbler songs uttered in 2016, in response to playback songs of six different populations. See main text for details.

	Frequency (Hz)				Length (seconds)			
	Min 5%	**Response to**	**Max 95%**	**Response to**	**Min**	**Response to**	**Max**	**Response to**
Santiago	1033.6	LSW	4478.9	SN	0.211	ST	2.494	GSW
Fogo	1033.6	FG, SN, LSW, SW	4478.9	ST	0.143	FG	2.347	LSW
S. Nicolau	1033.6	ST	4823.4	SN	0.118	FG	2.691	SN



Supplementary figure S3.5 Boxplots of the score of the first principal component of the spectrogram measurements of songs uttered by the Cape Verde warblers, on the three existing populations, in response to songs of six different populations of origin; ST = Santiago, FG = Fogo, SN = S. Nicolau, GSW = greater swamp warbler, SW = Seychelles warbler, LSW = lesser swamp warbler.

Table S3.8 Principal component analysis of songs uttered in response to songs of different populations of origin on the three populations of the Cape Verde warbler; loadings for each of the three populations; absolute values > 0.5 are highlighted in bold.

		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Santiago	Aggregated entropy							0.433	**0.901**
	Average entropy						0.100	**0.896**	-0.432
	Q1 frequency	-0.226	**0.576**	-0.181	**0.501**	**0.577**			
	Q3 frequency	**-0.512**	0.160	0.442	**-0.638**	0.331			
	Center frequency	-0.338	0.464	0.328	0.248	**-0.708**			
	5% frequency	-0.130	0.344	**-0.771**	-0.465	-0.233			
	95% frequency	**-0.745**	**-0.556**	-0.264	0.255				
	Delta time						**-0.995**		
Fogo	Aggregated entropy							**-0.713**	**-0.696**
	Average entropy						0.157	**-0.680**	**0.717**
	Q1 frequency	-0.231	**0.565**	-0.202	-0.294	**0.708**			
	Q3 frequency	**-0.524**		**0.500**	**0.658**	0.200			
	Center frequency	-0.364	0.463	0.374	-0.441	**-0.565**			
	5% frequency	-0.136	0.423	**-0.662**	0.474	-0.374			
	95% frequency	**-0.722**	**-0.534**	-0.362	-0.249				
	Delta time						**0.983**	0.174	
S. Nicolau	Aggregated entropy							-0.324	**-0.946**
	Average entropy						0.157	**-0.935**	0.318
	Q1 frequency	-0.276	**0.578**	0.208	-0.381	**0.634**			
	Q3 frequency	**-0.544**		**-0.715**	0.364	0.244			
	Center frequency	-0.366	0.424	-0.177	-0.379	**-0.716**			
	5% frequency	-0.172	0.432	0.439	**0.751**	-0.162			
	95% frequency	**-0.682**	**-0.546**	0.470	-0.122				
	Delta time						**0.988**	0.145	

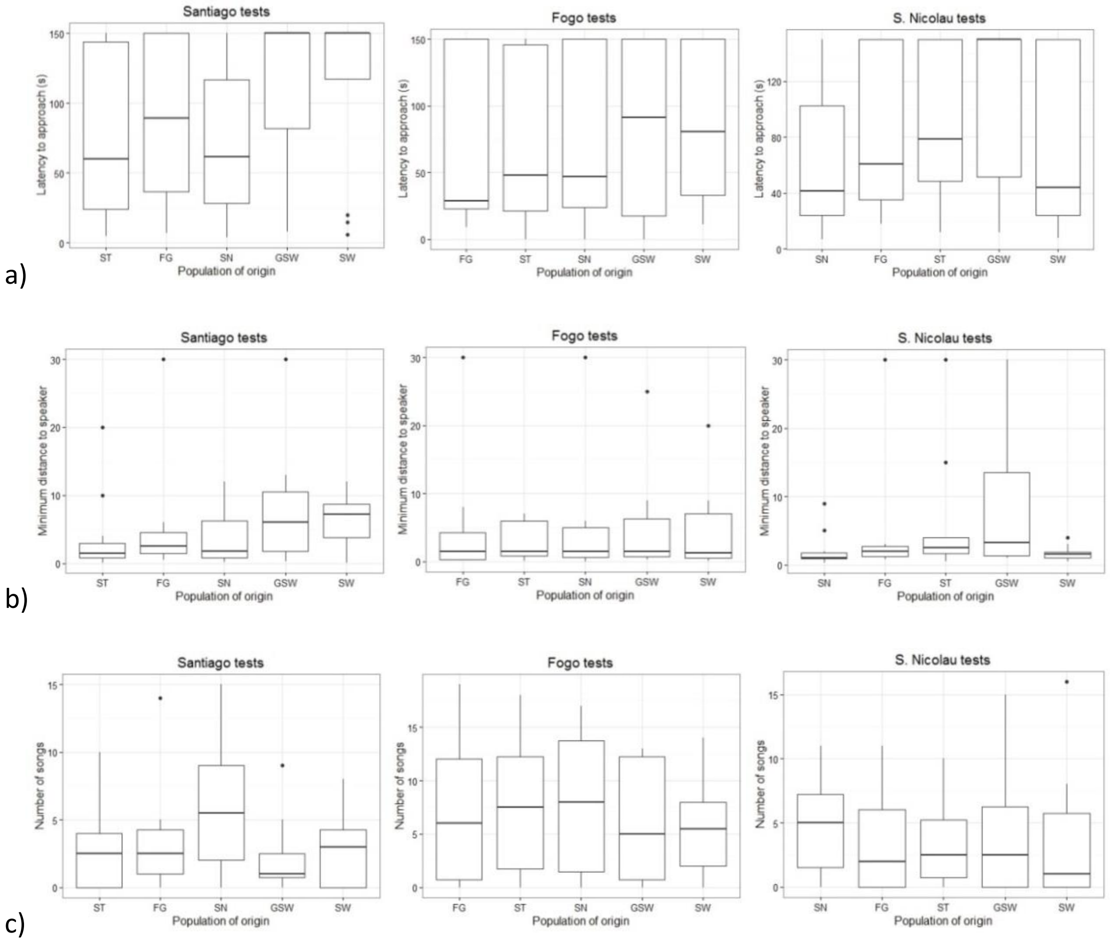


Figure S3.6 Boxplots of a) latency to approach, in seconds; b) minimum distance to speaker, in centimetres; and c) number of songs of the Cape Verde warblers, on the three existing populations, in response to songs of the five different populations of origin; ST = Santiago, FG = Fogo, SN = S. Nicolau, GSW = greater swamp warbler, SW = Seychelles warbler.

Supplementary table S3.9 Model selection table for the latency to approach within 5m, modelled with cox mixed models; minimum distance to speaker and number of songs, modelled with linear mixed models. Significant differences between full and reduced models, and corresponding delta AIC > 4 and best model in bold.

		model selection			**AIC**		**AIC df**	
		Delta AIC	**Chisq**	**p**	**Reduced**	**Full**	**Reduced**	**Full**
Latency to approach	Santiago	2.012	1.454	0.483	222.397	224.409	13.651	15.655
	Fogo	**9.489**	**10.991**	**0.004**	252.475	**242.986**	12.344	14.982
	S. Nicolau	**7.62**	**9.309**	**0.01**	269.195	**261.576**	12.237	14.819
Minimum distance	Santiago	1.99	2.014	0.365	363.43	365.42	7	9
	Fogo	**4.61**	**8.605**	**0.014**	382.68	**378.07**	7	9
	S. Nicolau	1.85	2.155	0.34	341.18	343.03	7	9
Number of songs	Santiago	2.86	1.142	0.565	326.91	329.77	7	9
	Fogo	0.65	3.343	0.188	358.52	359.17	7	9
	S. Nicolau	1.41	2.591	0.274	346.02	347.43	7	9

Chapter 4

Habitat preferences in the Cape Verde warbler *Acrocephalus brevipennis* and implications for conservation

Main habitat of the Cape Verde warbler Acrocephalus brevipennis *on S. Nicolau: invasive reed* Arundo donax *stands, most of which are at risk of being cut down for agriculture. Photo taken in September 2014.*

4.1 Abstract

Understanding habitat preferences of endangered species is important for conservation, as it provides a geographical framework for management actions to be applied. Here, I assessed habitat preferences of the endangered Cape Verde warbler *Acrocephalus brevipennis* on the three islands where it exists. At a landscape scale, satellite images were used to create maps of the areas of habitat that were used within each island to assess if key predictors could be identified. The presence of green vegetation cover that lasted throughout the dry season, assessed from the NDVI calculated from satellite images, was the best predictor of warbler presence at this large scale. At a local scale, habitat plots were directly sampled to assess key differences between sites used or not by the warbler during the breeding season on the three different islands. Neither coarse nor fine structural habitat features predict warbler presence within areas of apparently suitable habitat. However, certain plant species predicted warbler presence, and these key plant species differed between plots used by the warbler on the three islands. Importantly, landscape and local scale analyses together indicate that the small population on the island of S. Nicolau does not appear to be using the entire putatively suitable habitat, but the reason for this is unknown. These results provide important information regarding key habitat requirements that appear to determine or limit the Cape Verde warbler's distribution. I therefore provide a basis for identifying and surveying areas suitable for warblers across the islands, and information on which to base practical habitat conservation measures to help protect and expand the remaining warbler populations.

4.2 Introduction

Habitat protection is critical to the conservation of endangered species (Committee on Scientific Issues in the Endangered Species Act *et al.* 1995; Dudley 2008; Lausche & Burhenne-Guilmin 2011). Protection and management of areas of relevant habitat are often identified as priorities for the conservation of threatened animals (Boyd *et al.* 2008; Donald *et al.* 2013; Dudley 2008). Assessing which factors determine habitat selection by endangered species is therefore crucial for effective and directed conservation (Manly *et al.* 1993). For rare or cryptic species, the full extent of occupied areas is often unknown and information on habitat preferences is sometimes limited to occasional records (Buchanan *et al.* 2011; Howland *et al.* 2016; Rebelo & Jones 2010; e.g. Svensson *et al.* 2010; Zeng *et al.* 2015; Zhao *et al.* 2017). This makes efficient conservation actions difficult to determine, but a combination of field data and modelling can be used to effectively predict the full extent of areas and habitats occupied. Information on habitat may inform conservation opportunities, for example when a strong association of a species with a certain habitat feature is revealed (Eglington *et al.* 2008; Howland *et al.* 2016; VanderWerf *et al.* 2016). Thus it is important that the full extent of the area occupied by such species and their habitat preferences are assessed before conservation plans can be put into action.

Ecological processes that determine habitat properties have been shown to change with spatial scale (Turner 1989). The factors that influence species distribution can drive habitat preferences similarly at large *landscape* and fine *local* scales (Brambilla *et al.* 2009; Holbrook *et al.* 2017; Illera *et al.* 2010). However, in many cases different factors play a role at different spatial scales (Border *et al.* 2017; Howland *et al.* 2016; Wiens *et al.* 1987). For example, for many birds, topographic features or vegetation cover can be important at landscape scales (Border *et al.* 2017; Irvin *et al.* 2013), but at local scales other features such as predation risk or presence of specific plants can be determinants (Border *et al.* 2017; Chalfoun & Martin 2007). It is also important to understand if different environmental factors are playing different roles in habitat selection across regions, but such studies are surprisingly rare (Fielding & Haworth 1995; Morris *et al.* 2001; Rodríguez & Andrén 1999; Whittingham *et al.* 2003). In one of the few studies undertaken to date, differences in nesting habitat of birds of prey between island and mainland regions in Scotland have been speculated to be linked to habitat availability (Fielding & Haworth 1995). However, when applied to other areas, there was a lack of generality in the predictive success of regional models (Fielding & Haworth 1995). This implies that caution must be exercised when applying predictions of species distribution models (SDMs) to different regions. However, they remain very useful conservation tools, used commonly in predicting occupancy, and gross scale habitat association studies that are often built to inform further specific study (Elith *et al.* 2006; Elith & Leathwick 2009). Similarly, it is important to consider whether a species is a habitat specialist or generalist. Generalist species successfully use a wide variety of environmental conditions and resources, whereas specialist species can only exploit a narrow range of conditions (Townsend *et al.* 2003). Thus, a generalist might be able to easily adapt to spatially or temporally heterogeneous habitats, while a specialist might be at risk of extinction if there are great changes in environmental conditions (Townsend *et al.* 2003). Understanding if a species is able to explore a wide variety of environmental conditions, particularly across different regions, or is constrained by very specialised habitat needs is crucial for its successful conservation (Devictor *et al.* 2008).

Determining the extent of the areas occupied by species via field surveys can be challenging, especially if the species are rare or cryptic (Buckland *et al.* 2000; Rebelo & Jones 2010; Vine *et al.* 2009), or if the potentially suitable areas are inaccessible (Bibby *et al.* 1992; Hill *et al.* 2005). To overcome this problem, SDMs are often employed (Bradie & Leung 2016; Elith *et al.* 2006; Elith & Leathwick 2009). SDMs aim to predict species distributions in geographical space based on their known distribution and inferred likely environmental drivers of habitat preferences (Bradie & Leung 2016; Elith & Leathwick 2009). The extent to which SDMs will reflect the actual species distribution depends on many factors (Elith *et al.* 2006; Elith & Leathwick 2009; Rebelo & Jones 2010). These factors include the type of environmental features used, the quality and resolution of the data, the extent of species distribution records, and the complexity of the relationships between the species and its environment, including competitor, parasite and predator interactions (e.g. Elith & Leathwick 2009; Godsoe *et al.* 2016; Guisan *et al.* 2013). If SDMs are accurate, they can undoubtedly be a useful tool in assessing population distribution and especially in situations with few data or difficult terrain (Guisan *et al.* 2013).

The Cape Verde warbler *Acrocephalus brevipennis* is a passerine endemic to the Cape Verde islands (Figure 4.1), currently classified as endangered (BirdLife International 2017). Currently, it is only present on Santiago, Fogo and S. Nicolau (Batalha *et al.* 2017). The three islands are geographically different from each other (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986). Since the colonisation by Europeans, ca. 500 years ago, the archipelago lost most of its natural flora, possibly as a result of anthropogenic effects and droughts (Hazevoet 1995). The current flora is dominated by introduced exotic plant species, particularly agricultural crops and planted forests (Arechavaleta *et al.* 2005; Hazevoet 1995). The warbler seems to inhabit somewhat different habitats on the three islands (BirdLife International 2016; Garcia-del-Rey 2016; Hazevoet 1995). Currently, on S. Nicolau, it is restricted to abandoned reed *Arundo donax* patches on the central area of the island and valleys with *Mangifera indica* mango trees (Batalha *et al.* 2017; Donald *et al.* 2004; Hazevoet *et al.* 1999). On Santiago, it was observed on sugarcane *Saccharum officinalis* and other coastal plantations and on central vegetated areas in the 1980s (Hazevoet 1995), and is currently present in a variety of densely vegetated areas (Batalha *et al.* 2017). On Fogo, the bird was discovered in 2004 on coffee *Coffea arabica* plantations interspersed with maize and fruit trees (Hering & Fuchs 2009; Hering & Hering 2005). Overall, it seems to inhabit densely vegetated and somewhat irrigated or wet areas (Cramp & Perrins 1992; Garcia-del-Rey 2016; Hazevoet 1995). The fact that it has been reported using different habitats on different islands (Donald *et al.* 2004; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Hering 2005) suggest its habitat preferences are related to structural features which are provided by different types of available vegetation on each island are.

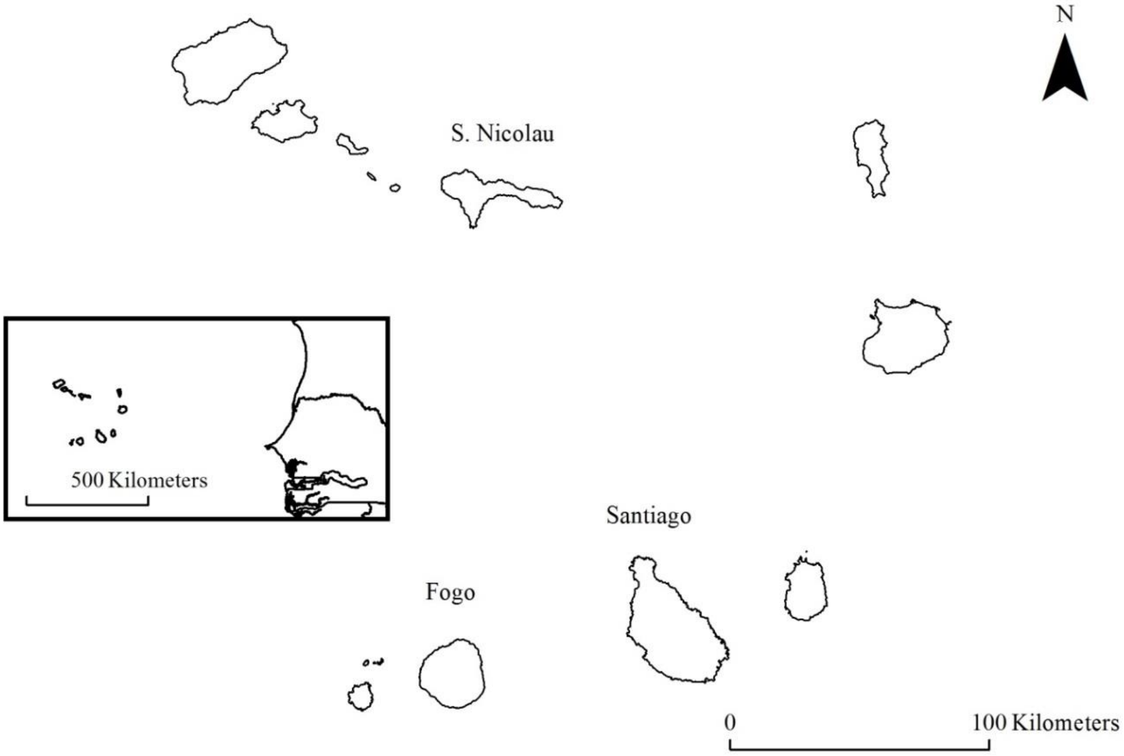


Figure 4.1 Map of the Cape Verde islands (main) with position relative to West Africa (inset). Cape Verde warbler populations currently exist on Santiago, Fogo and S. Nicolau. *Map produced using open source data on ArcMap 10.1 by Helena Batalha.*

Here, I used vegetation and habitat data, remotely sensed and collected in the field, to assess drivers of distribution and divergence in the three populations of the Cape Verde warbler. The aims were to: 1) assess if the species distribution at a landscape scale is determined by evergreen dense vegetation cover and produce predictive distribution maps; 2) investigate associations between habitat use and vegetation structure at a local scale; and 3) determine potential differences in habitat use between islands.

4.3 Materials & Methods

4.3.1 Landscape scale: predicting area of occurrence of the Cape Verde warbler

I built a SDM to assess if the continuous presence of dense green vegetation cover over both the wet and dry season could be used to predict areas of occurrence of the warbler on the three islands. Spatial data were analysed with ArcMap 10.1 (ESRI 2011) and MaxEnt (Phillips *et al.* 2006; Phillips *et al.* 2004). To my knowledge, vegetation type surveys in Cape Verde are limited to the terrestrial protected areas (Vasconcelos *et al.* 2012) which make up 2.6% of the total country area (The World Bank 2014), and there is no complete land coverage survey at a national level. However, it is possible to assess vegetation cover from satellite images. The normalised difference vegetation index (NDVI) is a reflectance based index commonly used to indicate the amount of dense, green vegetation in an area, and is a widely used layer in geographic information system based distribution studies (Carlson *et al.* 1990; Pettorelli *et al.* 2005). It is based on the difference in the quantity of light absorbed and reflected by live vegetation in the visible and near-infrared regions, and it ranges between -1 and 1 (Carlson *et al.* 1990). The more green leaves there are in an area, the higher the NDVI value will be. Oceans and clouds will produce NDVI values <0; barren areas will show low NDVI values, typically -0.1 to 0.2 (Carlson & Ripley 1997). Sparse vegetation such as that found in savannas or grasslands will result in medium NDVI values (0.2 to 0.4); and densely vegetated areas such as tropical forests, will yield the highest NDVI values, close to 1 (Carlson & Ripley 1997). Since NDVI is calculated from satellite images, cloud cover can be an issue as it can artificially decrease its value (Pettorelli *et al.* 2005). Therefore, when calculating it, it is best to use images free from cloud cover (Pettorelli *et al.* 2005).

To calculate NDVI values, composite images were downloaded from the dataset L8 OLI/TIRS, accessible at https://earthexplorer.usgs.gov/. For Fogo I used images of path 201, row 50 and for Santiago and S. Nicolau I used images from path 210, row 49. Of all the datasets available, L8 OLI/TIRS was the only one with images at a sufficient spatial resolution for my analysis (30x30m). NASA's Landsat 8 satellite collects images of Earth each 16 days, and since oceanic islands are often covered in clouds, I used only one image per season (the one with the least cloud coverage taken on the same date for both sets of islands). I used a satellite image representative of the dry season (April to June) and one representative of the wet season (September to November) in the Cape Verde archipelago (Correia 1996; Hazevoet 1995). For Fogo I used images LC82100502013245LGN00 (wet season, 02/09/2013) and LC82100502014136LGN00 (dry season, 16/05/2014); for ST and SN we used LC82100492013245LGN00 (wet season, 02/09/2013) and LC82100492014136LGN00 (dry season, 16/05/2014). I downloaded files in the GeoTIFF format, including all the spectral bands and geographic referencing. I then used ArcMap 10.1 to calculate NDVI values from bands 1 to 6, and saved the data in ASCII format to use in MaxEnt version 3.3.3k (Phillips et al., 2006, Phillips and Dudík, 2008).

To predict potential suitable habitat for the Cape Verde warbler on the three islands, I used MaxEnt, a software that uses presence data only (no absence data) and environmental layers to predict potential distribution maps for given species (Phillips *et al.* 2004). MaxEnt has been increasingly used to predict species distributions (Bariotakis *et al.* 2016; Border *et al.* 2017; Illera *et al.* 2010; Zhao *et al.* 2017), as it requires presence only data and performs well relative to other SDM methods, even with small sample sizes (Elith *et al.* 2006; Elith *et al.* 2011). It provides a continuous logistic output of the habitat suitability for the intended species given the selected environmental variables. I used the two NDVI values (wet season and dry season) and warbler presence data on Santiago, Fogo and S. Nicolau (from observations collected during the wet season of 2013) in MaxEnt to predict the area occupied by the warbler. I used presence data (GPS points) collected in 2013 by surveying sites where the warbler was known to be present from the literature (Donald *et al.* 2004; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Fuchs 2009) plus sites identified by my team in 2013 and where it had not previously been reported (Batalha *et al.* 2017). I used a total of 57 presences for Santiago, 42 for Fogo and 22 for S. Nicolau. I modelled the three datasets separately to account for potential differences in the relationships between warbler presence and vegetation cover across islands. To evaluate model fit and predictive ability, MaxEnt calculates a metric called “the area under the receiving operator characteristic curve”, AUC. This metric is an indication of the ratio of the model’s *sensitivity* (*true positive rate*, i.e. proportion of positive data points that are correctly considered as positive, with respect to all positive data points) to the model’s *specificity* (*1 - true negative rate*, which is equivalent to the *false positive rate*, i.e. the proportion of negative data points that are mistakenly considered as positive, with respect to all negative data points). The rationale behind this is to get a measure of the ratio of true versus (potentially) false presences. Therefore, the higher the AUC, the better the predictive ability of the model should be. Generally, an AUC value > 0.70 is considered to indicate a good discriminant ability of the model (Lobo *et al.* 2008; Pearce & Ferrier 2000), but this can only be verified with presence-absence data. It is important to note that MaxEnt does not deal with real absences, so the randomly selected points for which there was no real record are simply background points and species absence from those points might be real or just result from a lack of records (Lobo *et al.* 2008; Phillips *et al.* 2006). I assessed model fit by examining the AUC of each model and determined the influence of both predictor variables, i.e. NDVI value for wet and dry season, in the species' distributions by assessing their respective permutation importance (Maslo *et al.* 2016).

4.3.2 Local scale: habitat use by the Cape Verde warbler

Habitat sampling took place between September and November 2014 on Santiago, Fogo and S. Nicolau. I did not use sample habitat along a grid or transect because patches of vegetation that could potentially be occupied by the warbler appear rather irregularly throughout the Cape Verde islands (pers. obs.), and this can cause traditional censuses or regularly spaced point counts to fall outside potentially suitable habitat or in inaccessible locations (Batalha 2014). For these reasons, sampling along a grid or transect can cause observers to miss many suitable habitat points or individuals and thus strongly reduce the data points collected within my time and logistical constraints. This means that the sampling was not completely random, thus artificially increasing the probability of finding the birds. However, the alternative would be to risk not obtaining a large enough sample size. The Cape Verde warbler inhabits densely vegetated patches on all three islands (Donald *et al.* 2004; Hazevoet 1995; Hering & Fuchs 2009). Initial surveys confirmed that the warbler was absent from more barren areas. I then restricted habitat sampling to areas with structured vegetation (of any kind), including areas where the bird had been previously detected (Batalha *et al.* 2017; Donald *et al.* 2004; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Fuchs 2009), and areas with dense vegetation but where the bird had not been recorded before. Within those areas I sampled 10x10 m random habitat plots throughout accessible locations. I aimed to sample plots in all kinds of dense vegetation, e.g. coffee or sugarcane plantations, mountain or coastal forests, patches of abandoned reed, etc. This was done in as many different areas of each island as possible when taking into account logistic and time constraints. I aimed to sample a minimum of ten locations where the bird was present (positive locations) and five locations where the bird was absent (negative locations) per island in the available time.

At each location, I sampled a minimum of three 10x10 m plots (four plots at three locations) roughly 50 m away from each other, following Showler *et al.* (2002). I aimed to sample locations at least 200m away from each other, across the entire densely vegetated areas of the islands, but due to the narrow distribution range of the bird, difficult accessibility of habitat in some areas of the islands and very small population on S. Nicolau this was not always possible. The minimum distance between two independent sampling locations was 87 m for Santiago, 106 m for Fogo and 88 m for S. Nicolau. Before sampling geographically close locations, careful consideration was taken to ensure that 1) if birds were present, they held two independent territories or 2) the general habitat type (e.g. sugarcane plantation, forest, reed patches) differed between locations. The three islands where the Cape Verde warbler is present display a multitude of micro-habitats, created by different combinations of elevation, rainfall and vegetation type (Hazevoet 1995). The landscape can change dramatically over distances of less than 200 m (pers. obs.). In each plot, several structural vegetation traits and topographical features including elevation and slope were measured (see Table 4.1). Green vegetation needs abundant water, and in Cape Verde there are three main water sources: 1) the rainy season (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986); 2) the north-eastern trade winds, which bring mist clouds as they encounter the mountains, thus making the north-eastern slopes wetter than the rest of the islands (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986); and 3) by artificial irrigation and dams, which are widely used for agriculture (Hazevoet 1995). There are no permanent natural water sources on Cape Verde apart from a few streams on Santo Antão. For all these reasons, distance to water bodies was not included in the analyses as it unlikely to represent a real association with available water or soil moisture.

To determine the exact area to sample, I would select a random point, record it in a Garmin eTrex® H GPS and wait for 10 minutes (5 minutes of silence and 5 minutes of male song playback) to check if warblers would come within approximately a 14 m radius of where the GPS was; I would then mark a 10x10 m square encompassing the GPS and the point where the bird had landed. Playbacks were used because they increase detection rate (VanderWerf *et al.* 2016), and even though they are more likely to attract adult males for this study the age and sex of the birds observed did not matter. If birds were present, they would usually come within a 10 m radius; only on one occasion did a bird come less than 14m away but not less than 10 m. On a few occasions birds appeared after the initial 10 minutes period, i.e. while the vegetation sampling was being underway. In these cases I considered birds to be absent from the plot. The plots were positioned at least 50 m from each other, in different directions mainly determined by terrain accessibility and presence of vegetation. Habitat features measured within each plot (Tables 4.1 and 4.2) were selected based on literature (Braun-Blanquet 1932; Lee & Marsden 2008) and my own assessment of warbler territory features, as recommended by Bibby *et al.* (1992) and Wildi (2013). All percentage cover measurements were approximated to the nearest superior value within six categories based on the Braun-Blanquet scale (Braun-Blanquet 1932; Wikum & Shanholtzer 1978): 0%, 5%, 25%, 50%, 75% or 100%.

Table 4.1 Cape Verde warbler habitat variables measured within each 10x10 m presence or absence sampling plot during the breeding season of 2014, with corresponding unit, estimation method and description.

Variable	Unit	Estimation	Description
Elevation	metres	GPS	Altitude above sea level
Slope	degrees	visual	Terrain inclination
Maximum vegetation (type) cover	percentage	visual	Maximum percentage of ground area, in a horizontal plane, covered by a certain vegetation type (see Table 4.2)
Maximum bare ground cover	percentage	visual	Maximum percentage of ground area, in a horizontal plane, not immediately covered with any plant species (e.g. clear ground under mango and coffee trees)
Maximum shade cover	percentage	visual	Maximum percentage of ground area, in a horizontal plane, that could be shaded because of being covered by tree canopy, reed or shrub coverage
Maximum vegetation (type) height	metres	visual	Maximum height of each vegetation category, to the nearest metre
Average maximum vegetation height	metres	visual	Average of maximum vegetation height at five different points within the plot, regardless of vegetation type
Average diameter	centimetres	tape measure	Average diameter of 20 stems, branches and tree trunks representative of the most abundant plant species within each plot, measured at breast height (1.2 to 1.5 m)

Table 4.2 Definitions for vegetation categories measured within each 10x10 m sampling plot in this study of Cape Verde warbler habitat, with examples.

Vegetation category	**Examples**	**Definition**
Tree	Mango (*Mangifera indica*); coffee (*Coffea arabica*); mesquite (*Prosopis juliflora*)	Perennial plant with an elongated stem, or trunk, supporting branches and leaves in most species, having a reticulate structure but often at some distance from the ground
Reed	Reed (*Arundo donax*); sugar cane (*Saccharum officinallis*); maize (*Zea mays*)	Tall grass with slender often prominently jointed stems, having a vertical structure
Woody shrub	Spanish flag (*Lantana camara*); bushmint (*Hyptis pectinata*); Mauritius hemp (*Furcrarea foetida*); *Acacia* sprouts	Small to medium-sized woody plant, with multiple stems and usually shorter than a tree, having a reticulate structure that can start at ground level; because this classification was meant to reflect habitat structure and not species taxonomy, I included in this category sprouts of future trees and cactus-like plants
Herbaceous shrub	Morning glories (*Ipomoea* spp.); beans (*Phaseolus vulgaris*); other climbing plants (e.g. *Momordica charantia*)	Plants smaller than woody shrubs, with no persistent woody stem above ground, but providing a more structural layer of cover than herbs and grasses; this vegetation category was measured because in some places a large portion of the plot was covered in said plants, which meant that the warbler had plenty of place to hide

As there can be several layers of vegetation in one place, cover percentages can add up to more than 100%; shade cover is the only variable that represents the total foliage cover within a plot. Cape Verde has few native plant species, approximately 80 of which are endemic (Gomes *et al.* 2003), but many introduced species (Arechavaleta *et al.* 2005; Duarte *et al.* 2008; Hazevoet 1995). Plant species identification was done based on the latest biodiversity checklist published for Cape Verde (Arechavaleta *et al.* 2005), the official management plans for the Natural Parks of Serra Malagueta (Mason *et al.*), Monte Gordo (S. Nicolau) and Fogo, and with help of the local biologists. Within each plot I also visually estimated the maximum vegetation height at five different points and measured the diameter at breast height (ca. 1.30 to 1.50m) of 20 stems/trunks of the most representative vegetation species.

To test whether specific local scale features, within the larger densely vegetated areas, predict the presence of the warbler, I used logistic generalized linear mixed models with warbler presence as a response variable and location within island as a random effect. To avoid overparameterising the models I defined three subsets of variables to be tested in order

1. *Coarse features* representative of the general habitat structuring of each plot, including topography features (elevation, slope) and general composite vegetation structure features (average maximum vegetation height, bare ground cover and shade cover).

2. *Fine features* that contributed to the composite features previously examined (i.e. maximum height and percentage cover of each of the vegetation types) and average diameter of stems and branches at breast height.
3. Individual *plant species* cover; analyses were restricted to the 14 most common plant species found in at least ten plots, and with minimum average cover of 25% of the plot.

I tested for spatial autocorrelation between sampling plots with the function lm.morantest from package spdep (Bivand *et al.* 2013; Bivand & Piras 2015), and there was autocorrelation on all islands (all Moran's I ≥ 2.632, all p values ≤ 0.004). Therefore, I included location as a random effect in the final models. Percentage cover variables from each plot were modelled as continuous data (Border *et al.* 2017; Eglington *et al.* 2008; Smart *et al.* 2006). No multicollinearity within each subset was detected after testing with the function vif in package car (Fox & Weisberg 2011): all VIF between 1.152 and 1.477 for coarse features; 1.493 and 2.471 for fine features; 1.16 to 1.80 for plant species. For each subset I fitted all possible combinations of variables and used a model selection approach (Burnham & Anderson 2007) comparing each model's Akaike Information Criterion (AIC) as implemented in function dredge of package MuMIn (Bartoń 2009). Models that differ by ΔAIC < 2 are treated as equivalent (Burnham & Anderson 2007). To estimate the relative importance of the explanatory variables, I calculated model-averaged coefficients with the function model.avg of package MuMIn. I also compared the fit of the best model (with the lowest AIC) to the fit of the null model (intercept and random effects only) with AIC and another measure of goodness of fit, the pseudo-R^2 (Nakagawa & Schielzeth 2013). The pseudo-R^2 quantifies the variance explained by the fixed effects (marginal R^2) and fixed and random effects (conditional R^2). A good predictive model would not have a large difference between the two pseudo-R^2, reflecting that fact that the random effects do not account for much of the variance relatively to the fixed effects. I calculated the pseudo-R^2 with the function r.squaredGLMM (Johnson 2014; Nakagawa & Schielzeth 2013) of package MuMIn.

Finally, I verified if MaxEnt habitat suitability at a landscape scale predicted warbler presence at a local scale. In other words, if within densely vegetated areas, warblers were present in sites with higher dense evergreen vegetation values and absent from sites with lower values. I overlaid the results of the habitat sampling points of 2014 to the MaxEnt output maps (Figures 4.2 to 4.4) and extracted the habitat suitability value for each point. As previously, location was included as a random effect in the final model. I used a binomial logistic mixed model with NDVI as a predictor and island as a factor, using function glmer, package lmertest, adapting the method used by Border *et al.* (2017).

To test whether the warbler uses different habitats across the islands, I performed a principal components analysis (PCA) for each subset of variables (coarse, fine and plants) using prcomp of package stats (R Core Team 2016). I used only plots where the warbler was present. For plant species I ran an additional PCA using presence and absence data. The purpose of this was to compare plant species composition in plots used by the warbler (where it was present) and plant species composition of the available habitat overall (plots where the warbler was present and plot from where it was absent). I also tested for significant differences between groups (islands) using the presence-absence data and the presence only data with a MANOVA with function Wilks.test, package rrcov (Todorov & Filzmoser 2009). For easier visualisation of plot clusters, I included one confidence interval ellipse per island on the PCA plots, created with the function autoplot of package ggplot2 (Wickham 2009). The ellipses show the 0.95 confidence interval assuming a normal distribution (Fox & Weisberg 2011).

4.4 Results

4.4.1 Landscape scale: predicting area of occurrence of the Cape Verde warbler

MaxEnt models based on remotely sensed data predicted the larger areas of distribution of the Cape Verde warbler. The habitat suitability maps produced with MaxEnt (Figures 4.2 to 4.4), which broadly describe the greenness and density of the vegetation on the Cape Verde islands, had the following AUCs: Santiago = 0.866, Fogo = 0.988 and S. Nicolau = 0.980. The NDVI of the dry season contributed more to predicting habitat suitability (Table 4.3), suggesting that areas with year round dense green vegetation best predicted warbler presence. However, on S. Nicolau, the importance of NDVI was lower than on other islands (Table 4.3).

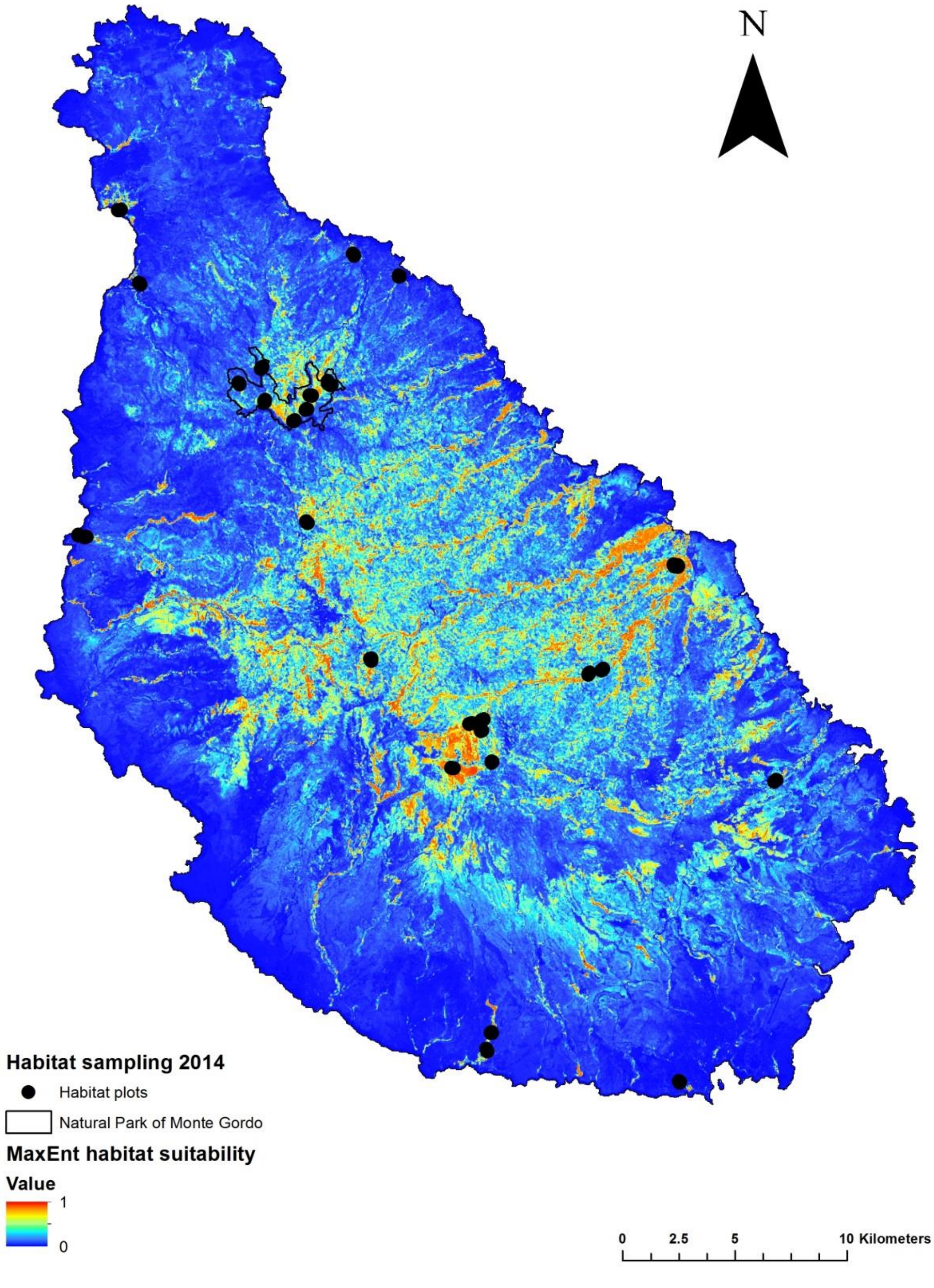


Figure 4.2 Predicted area of occurrence of the Cape Verde warbler for the island of Santiago, based on the proxy for vegetation cover, NDVI, of the wet season (September 2013) and dry season (May 2014); most areas with higher suitability values are either forested mountain tops of artificially irrigated plantations in dry riverbeds; habitat sampling plots (November 2014); area of the Natural Park of Serra Malagueta overlaid.

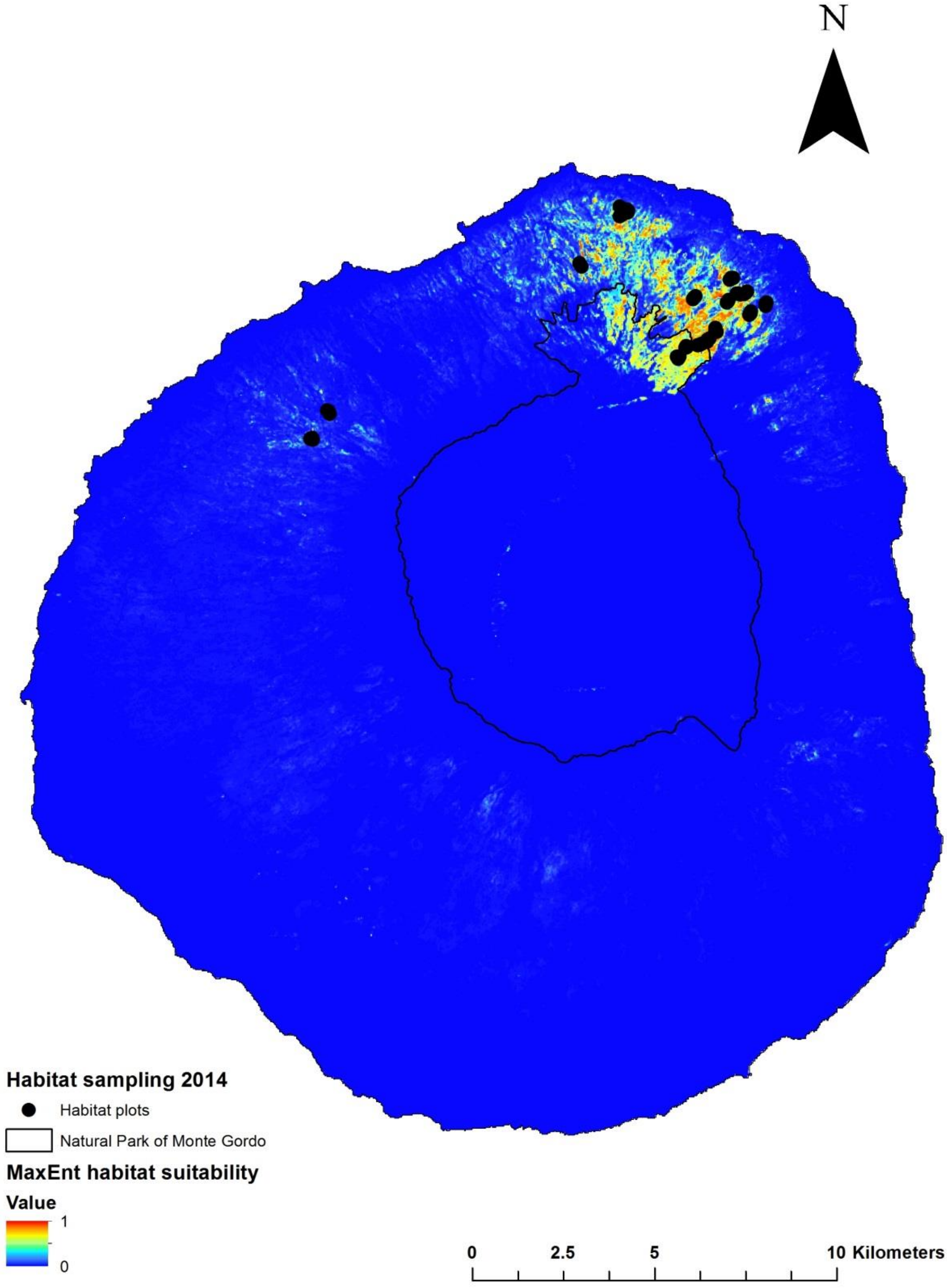


Figure 4.3 Predicted area of occurrence of the Cape Verde warbler for the island of Fogo, based on the proxy for vegetation cover, NDVI, of the wet season (September 2013) and dry season (May 2014); most areas with higher suitability values are either the forested area or the coffee plantations on the north-east slope of the island; habitat sampling plots (October 2014); area of the Natural Park of Fogo overlaid.

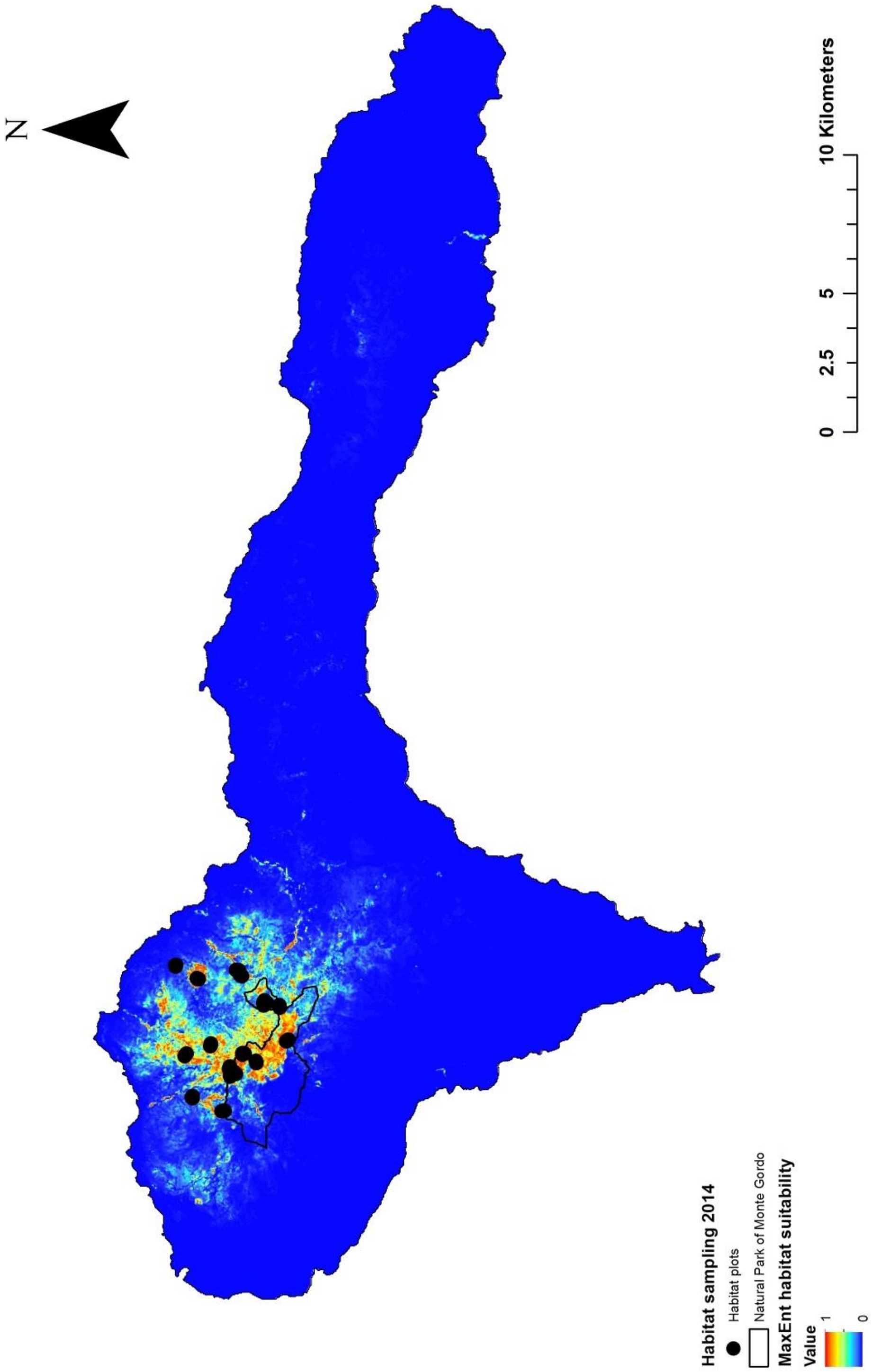


Figure 4.4 Predicted area of occurrence of the Cape Verde warbler for the island of S. Nicolau, based on the proxy for vegetation cover, NDVI, of the wet season (September 2013) and dry season (May 2014); most areas with higher suitability values are either the forested area or the central valley which retains moisture brought by the north-eastern trade winds; habitat sampling plots (September and November 2014); area of the Natural Park of Monte Gordo overlaid.

Table 4.3 Habitat suitability model for the Cape Verde warbler on the islands of Santiago, Fogo and S. Nicolau; this table shows the percent contribution and permutation importance of the variables used in the MaxEnt models (NDVI of dry and wet season) on each island.

Island	Variable	Percent contribution	Permutation importance
Santiago	Dry season NDVI	94.5	95.2
	Wet season NDVI	5.5	4.8
Fogo	Dry season NDVI	88.9	97.8
	Wet season NDVI	11.1	2.2
S. Nicolau	Dry season NDVI	62.6	73.4
	Wet season NDVI	37.4	26.6

4.4.2 Local scale: habitat selection by the Cape Verde warbler

I sampled a total of 192 habitat plots, in 63 independent locations across the three islands: 88 plots on Santiago, 52 on Fogo and 52 on S. Nicolau. The warbler was present in 102 plots and absent from 90. I identified 69 different plants (60 of them were identified to the species level, eight to the genus level and one to the family level). The 14 most common plants, i.e. the ones occupying an average of more than 25% of the plot area in at least 10 plots, are shown in Table 4.4. No plant species native to Cape Verde were among the most common in the sampling plots (Table 4.4).

Table 4.4 Plants species included in the analysis of Cape Verde warbler habitat, with number of plots where they were found and average percentage cover if present; *Mimosacea* refers to unidentified trees of this family.

Plant species	Plant type	Number of plots	Average percentage cover in plot
Prosopis juliflora	Forestry tree	18	69.72
Saccharum officinalis	Agricultural crop	36	66.94
Jacaranda mimosifolia	Forestry tree	10	53.00
Mangifera indica	Fruit tree	34	51.47
Coffea arabica	Agricultural crop	33	48.33
Eucalyptus spp.	Forestry tree	31	48.23
Arundo donax	Exotic	50	47.10
Lantana camara	Exotic	100	43.20
Musa spp.	Agricultural crop	25	38.80
Zea mays	Agricultural crop	48	34.79
Grevillea robusta	Forestry tree	17	29.12
Mimosaceae	Forestry tree	34	26.03
Pinus spp.	Forestry tree	10	26.00
Hyptis pectinata	Exotic	39	25.51

Coarse features

The best model (lowest AIC) for warbler presence with coarse features as explanatory variables retained only bare ground cover and average maximum vegetation height (Table 4.5). Model averaging indicates that average maximum vegetation height and bare ground cover are the variables that have the largest importance (0.67 and 0.66 respectively) in the subset of best models, with both present in five of the eight best models (Tables 4.5 and S4.1). The ΔAIC between the null and the best models is 1.7, and the conditional pseudo-R^2, is much larger than the marginal pseudo-R^2, (Table 4.6). This indicates that the random effect, ‘location’, has a disproportionate effect in explaining warbler presence when compared to the other variables tested. Additionally, the differences in AIC and pseudo-R^2 between the best model and the null model are small (ΔAIC < 2). This indicates that the coarse variables included in this analysis have little power to predict warbler presence within densely vegetated areas.

Table 4.5 Logistic linear mixed models for Cape Verde warbler presence with habitat features (coarse and fine features and plants) as predictors and location as a random effect. For the best model (lowest AIC), the table shows the estimate and standard error (SE), Z value test of significance and P value for each variable. For the models retained by the averaging procedure (ΔAIC ≤ 2 when compared to the best model) the table shows the coefficients, relative importance in the subset of models and number of models in which each variable was present. Significant values in bold.

		Best model				**Model averaging**		
		Estimate	**SE**	**Z**	**P value**	**Coefficients**	**Importance**	**N models**
Coarse features	**Intercept**	0.587	0.708	0.829	0.407	0.585		8
	Bare ground cover	-0.020	0.011	-1.838	0.066	0.0971	0.67	5
	Average maximum vegetation height	0.155	0.087	1.780	0.075	-0.0128	0.67	5
	Elevation					-0.0001	0.11	1
	Shade cover					-0.0323	0.22	2
	Slope					-0.0008	0.09	1
Fine features	**Intercept**	**-2.817**	**1.349**	**-2.087**	**0.037**	-1.946		22
	Average diameter	-0.043	0.042	-1.015	0.310	-0.001	0.04	1
	Maximum reed height	0.346	0.202	1.708	0.088	0.355	0.91	20
	Maximum woody shrub height	-0.631	0.391	-1.615	0.106	-0.183	0.33	7
	Maximum tree height	0.076	0.073	1.043	0.297	0.013	0.19	5
	Reed cover	0.025	0.013	1.858	0.063	0.012	0.53	11
	Tree cover	**0.029**	**0.014**	**2.003**	**0.045**	0.018	0.78	17
	Woody shrub cover	**0.027**	**0.013**	**2.063**	**0.039**	0.014	0.65	14
	Herbaceous shrub cover					-0.008	0.38	8

Table 4.5 (Cont.)

		Best model				Model averaging		
		Estimate	**SE**	**Z**	**P value**	**Coefficients**	**Importance**	**N models**
Plants	**Intercept**	**-1.855**	**0.657**	**-2.822**	**0.005**	-1.868		24
	Arundo donax	**0.057**	**0.019**	**3.025**	**0.002**	0.058	1	24
	Coffea arabica	**0.08**	**0.026**	**3.043**	**0.002**	0.083	1	24
	Eucalyptus spp.	**0.043**	**0.021**	**2.038**	**0.042**	0.042	1	24
	Jacaranda mimosifolia	0.06	0.038	1.596	0.111	0.051	0.82	19
	Saccharum officinalis	**0.027**	**0.011**	**2.463**	**0.014**	0.028	1	24
	Zea mays					-0.008	0.33	8
	Hyptis pectinata					0.007	0.31	8
	Grevillea robusta					0.008	0.27	7
	Mangifera indica					0.003	0.19	5
	Unidentified mimosaceae					-0.003	0.11	3
	Pinus spp.					-0.008	0.1	3
	Musa spp.					0	0.03	1

Table 4.6 Pseudo-R^2, AIC values and differences between the best and null models (ΔNull) for Cape Verde warbler presence with coarse features, fine features and plants as predictors. For the null model, pseudo-R^2 marginal is 0.00, pseudo-R^2 conditional is 0.67 and AIC is 228.8 for all variable subsets.

	Coarse		Fine		Plants	
	Best model	**ΔNull**	**Best model**	**ΔNull**	**Best model**	**ΔNull**
pseudo-R^2 marginal	0.047		0.118		0.319	
pseudo-R^2 conditional	0.696	0.027	0.736	0.067	0.777	0.107
AIC	227.1	1.7	226.9	1.9	205.4	23.4

Fine features

The best model (lowest AIC) for warbler presence with fine features as explanatory variables retained tree and woody shrub cover as significant variables (Table 4.5). Model averaging shows that maximum reed height and tree cover are the variables that have the largest importance (0.91 and 0.78 respectively) in the subset of best models, and both are present in 20 and 17 of the 22 best models (Tables 4.5 and S4.2). Woody shrub cover and reed cover are next, with an importance of 0.65 and 0.53 respectively, and are present in 14 and 11 of the 22 best models. This is consistent with the significant variables retained in the best model, suggesting that these are the variables that best explain warbler presence at a local scale. The conditional pseudo-R^2 is much larger than the marginal pseudo-R^2 for the best model, and the differences in AIC and marginal pseudo-R^2 between the best model and the null model are small (ΔAIC < 2), even if slightly larger than for the model with the coarse features (Table 4.6).

This suggests that none of the fine variables has great power in predicting warbler presence within densely vegetated areas, but there might be a tendency for the warbler to associate with certain structural features.

Plants

The best model (lowest AIC) for warbler presence with plants as explanatory variables retained percentage cover of reed, coffee, eucalypt *Eucalyptus* spp. and sugarcane (Table 4.5). Model averaging shows that reed, coffee, eucalypt and sugarcane are the plants that have the largest importance (all = 1) in the subset of best models, and all are present in the 24 best models (Tables 4.5 and S4.3). These are the same significant variables retained in the best model, suggesting that these are the variables that best explain warbler presence at a local scale. The conditional pseudo-R^2 is much larger than the marginal pseudo-R^2 for the best model but, unlike with the coarse and fine features models, the differences in AIC and marginal pseudo-R^2 between the best model and the null model are large (Table 4.6). The ΔAIC is 23.4, and the difference in variance explained by the best model and the null model is 0.319 (Table 4.6). This suggests that the model with these plant percentage cover explains warbler presence at a local scale significantly better than the null model.

At a local scale, warbler presence was not significantly more likely at points with higher habitat suitability in the MaxEnt output for any island (Table 4.7).

Table 4.7 Logistic generalized linear model for Cape Verde warbler presence on each island with NDVI based MaxEnt habitat suitability as predictor and location as a random effect. The table shows the estimate and standard error (SE), the z value test of significance and P value for each island.

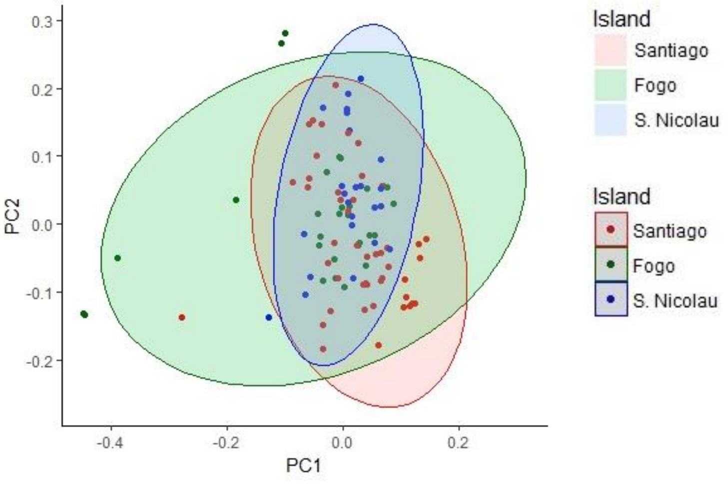
	Estimate	**SE**	**Z**	**P value**
Intercept	-0.551	0.766	-0.720	0.472
Santiago	1.581	1.437	1.100	0.271
Fogo	3.277	1.751	1.871	0.061
S. Nicolau	-0.219	1.716	-0.127	0.899

Differences across islands

For coarse and fine habitat features, neither of the PCAs suggested any clustering per island (Figure 4.5). In fact, the data points for all three islands overlapped largely in both PCAs (Figure 4.5). For the coarse and fine features the two first axes of the PCA combined explained 60.2%

and 58.1% of the variance respectively (Tables S4.4 and S4.5). None of the PCA axes for either of the coarse or fine features explained more than 38.8% of the variance (Tables S4.4 and S4.5). Wilks’ lambda was large for both habitat feature subsets, when using only presence data (Wilks' Lambda = 0.570, Chi^2-Value = 54.474, DF = 10, p-value < 0.001 for coarse features; Wilks' Lambda = 0.506, Chi^2-Value = 64.762, DF = 18, p-value < 0.001 for fine features).

For percentage coverage by plants, the first two axes of the PCA using only plots where the warbler was present explained only 29.6% of the variance, and none of the axes explained more than 17.7% of the variance (Tables S4.6 and S4.7). When plotted, this PCA shows a large reduction in variance on the S. Nicolau cluster of points when compared to the other two islands (Figure 4.5). Wilks’ lambda for the plant PCA using only plots where the warbler was present was small (Wilks' Lambda = 0.093, Chi^2-Value = 219.7, DF = 28, p-value < 0.001), indicating that between population variation in plants used is large when compared to within population variation. By contrast, in the plant PCA including both the plots where the warbler was present and absent did not show any reduction in the variance explained for S. Nicolau when compared to the other two islands (Figure 4.6). For the plant PCA using presence and absence data, Wilks’ lambda was relatively large (Wilks' Lambda = 0.309, Chi^2-Value = 214.23, DF = 28, p-value < 0.001), suggesting the between population variation is masked by within population variation. Reed, coffee, eucalypt and sugarcane coverage in plots where the warbler was present and absent show different trends on the three islands (Figure 4.7).



a)

Figure 4.5 Principal components analysis of a) coarse habitat features, b) fine habitat features and c) plants using plots where the Cape Verde warbler was present (n = 102), clustered by population. Ellipses show 95% confidence intervals.

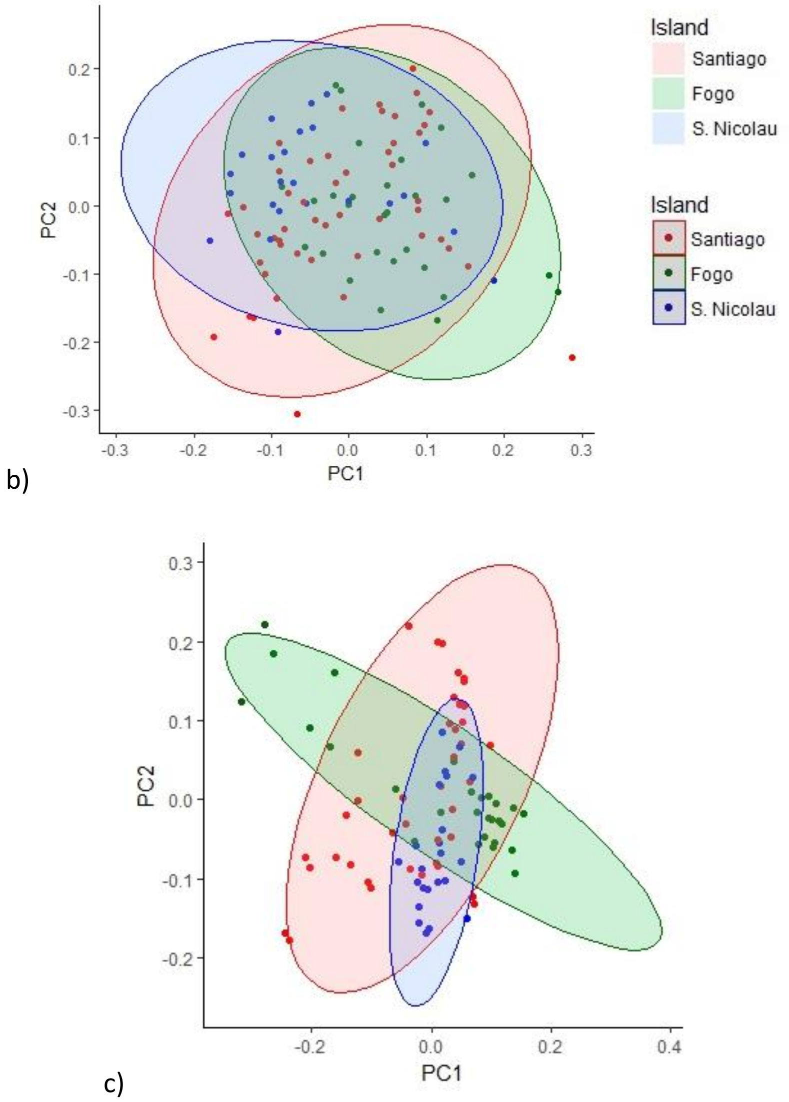


Figure 4.6 (Cont.)

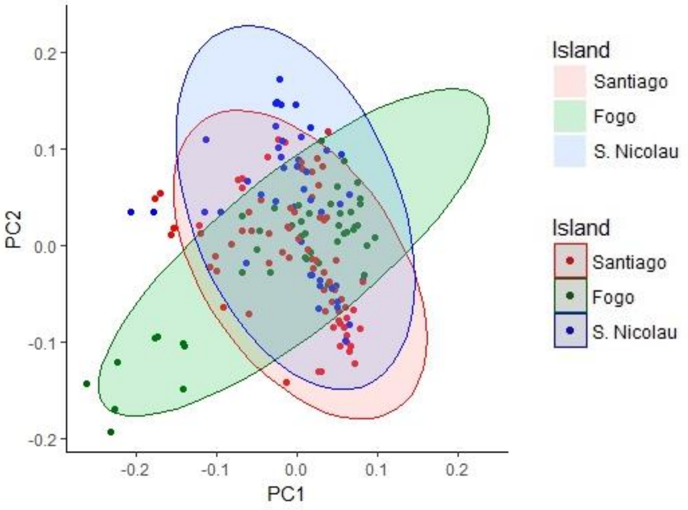


Figure 4.7 Principal components analysis of plant species composition using plots where the warbler was present and absent, for comparison (right; n = 192), clustered by population Ellipses show 95% confidence intervals.

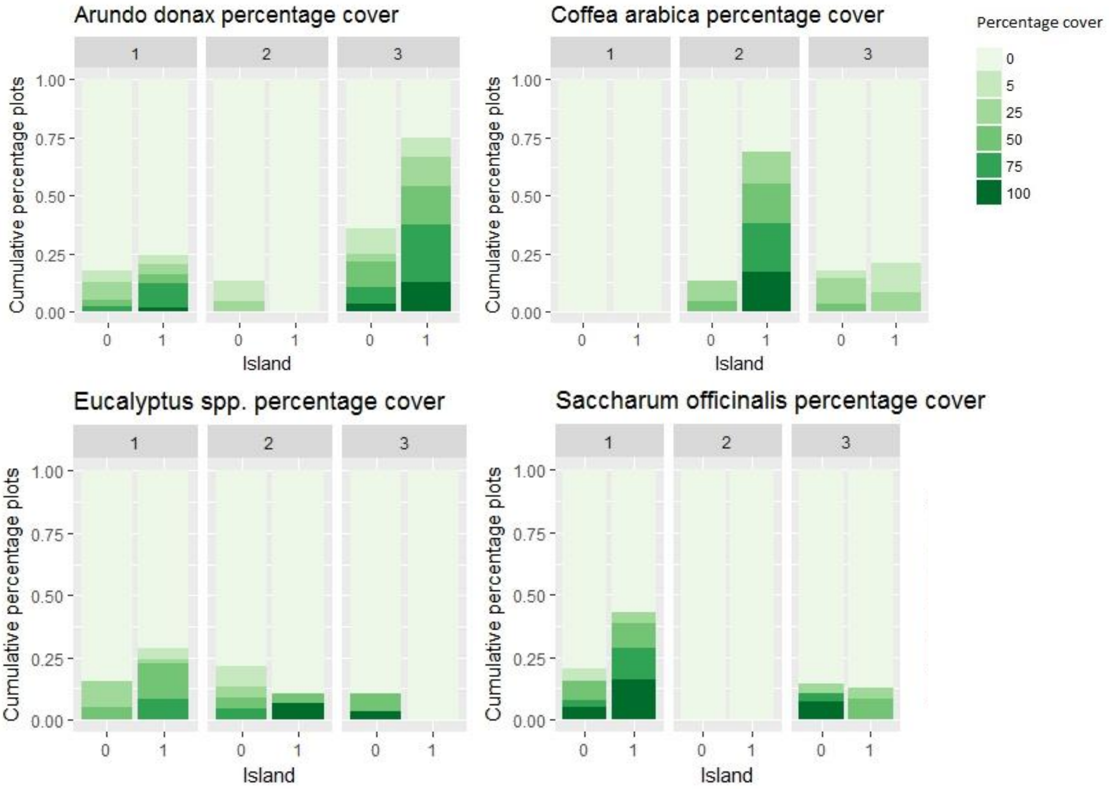


Figure 4.8 Barplots of number of plots with each percentage cover category of each species of plants that was significant in the models predicting warbler presence. Islands: 1 = Santiago; 2= Fogo; 3 = S. Nicolau. Within each island, plots where the Cape Verde warbler was present and absent are coded 1 and 0, respectively.

4.5 Discussion

Our study is the first to assess and compare the Cape Verde warbler habitat across the three islands where it exists. Overall, I found that the Cape Verde warbler can be generally found in evergreen, densely vegetated areas on Santiago, Fogo and S. Nicolau, but not in areas where the vegetation is not dense or green year round. However, neither the presence of dense green vegetation nor structural habitat characteristics predicted warbler presence at a local scale. There were no structural habitat differences in sites where the warbler was found between islands. However, there were differences in plant species composition in sites used by the warbler between S. Nicolau and the other two islands. Overall, this suggests that the warblers select their territories within densely vegetated areas based on something other than vegetation structure (e.g. prey abundance, nesting sites, absence of predators). It also suggests that the warblers of S. Nicolau use a more specialised habitat than the warblers on Santiago or Fogo. This could be related to a process or trait intrinsic to that population (e.g. they can be suffering from inbreeding depression).

MaxEnt models based on remotely sensed data predicted the larger areas of suitable habitat for the warbler on all three islands. The NDVI values (used as a proxy for the density of green leaves) in the dry season contributed to the output maps more than the NDVI during the wet (breeding) season. This suggests that the warblers might set up territories in areas where there is dense, green vegetation year round, even during the dry season. The Cape Verde warbler breeds after the first rains, during September to November (Cramp & Perrins 1992; Hazevoet 1995) and, at a first glance, it would seem plausible that the presence of green vegetation during the wet season would be important in determining their breeding habitat selection. However, this is not the case, as green vegetation during the dry season was more important in predicting areas of suitable habitat for the warbler, even though predictions were based on records of the warbler during the wet season (Table 4.3). To my knowledge the phenology of this bird has never been studied in detail (Cramp & Perrins 1992; Garcia-del-Rey 2016; Hazevoet 1995). However it is plausible that these birds hold the same territories during the whole year, as for example the Seychelles warbler *Acrocephalus sechellensis* (Komdeur 1992). Warblers are heard singing, probably in pair-bonding behaviour, in April and May during the dry season (A. Rendall, pers. comm.) and song playback tests performed during the dry season elicited territorial defence behaviour by the same colour-ringed birds identified in the location in the previous wet season (pers. obs.). It seems likely that the birds establish their territories during the dry season, when areas with green vegetation are most constrained, so that they can hold a territory during the whole year. The dense, evergreen vegetation might provide shelter from predators, abundant food supply and support structures for birds to build their nest on. Cape Verde, in the Sahel zone, has an arid, semi-tropical climate with a short rainy season (Correia 1996; Hazevoet 1995; Sena-Martins & Moreno 1986). The islands are located slightly north of the Intertropical Convergence Zone, which means that the rainy season might not occur every year (Correia 1996; Philander *et al.* 1996), and droughts of up to 18 years have been recorded (Hazevoet 1995). Distance to the nearest water source was not used as a variable in the analyses because, in Cape Verde, there are no permanent water courses except on the island of Santo Antão (Hazevoet 1995) and the main sources of irrigation are the rains, ocean mists and anthropogenic irrigation (see Methods). Thus, distance to the nearest dry riverbed is unlikely to represent an association of sampled sites with water features. Aspect is often included in environmental models but in many cases it was impossible to determine due to terrain irregularity. However, I do not exclude the possibility that water availability is important for the warbler. For example, water retention by the soil can be important not just for vegetation but also for insect and arthropod abundance.

At a local scale, none of the coarse structural habitat features that I tested predicted the presence of the warbler. The best model of predictor variables was not better at explaining warbler presence than the null model. Neither elevation nor slope had any significant effect in predicting warbler presence. Contrary to previous observations (Hazevoet 1995; Hering & Fuchs 2009), I noted that the warbler can be found on all elevations, from sea level to the top of mountains, as high as 1384 m on Fogo (data not shown). It can also be found on all types of terrain topography, from flat areas such as sugarcane plantations to very steep, inaccessible ravines covered in invasive species (pers. obs.). As long as there is vegetation cover, elevation and slope do not seem to be important for this bird. In the best model testing for the impact of fine vegetation features, tree and woody shrub cover were the only significant variables, while reed cover and maximum reed height were close to significant. However, the “reed” variables include various species i.e. reeds, sugar canes and maize, because all three plants have the same vertical structure, different from trees and shrubs. Model averaging indicated that these four variables (tree, woody shrub and reed cover and maximum reed height) had most importance in determining the presence of the warbler (Table 4.5). However, the null model was also included in the top model set. This can indicate that none of the structural variables that I measured are key variables involved in habitat selection by the warbler at local scales, or that the models have little power because of the limited number of independent data points per island. However, it is worth nothing that the model including plant species cover showed different results than the models including structural variables (see below), suggesting the results of all models are not consequences of little power of the models. The association of the warbler with structural habitat features might be mediated by another factor that I have not measured. For example, food or nesting site availability might be important for the warbler at a local scale, but the limited knowledge on the bird’s feeding and nesting habits made it impossible to collect these data in a consistent way. None of the coarse or fine scale structural habitat features models differed significantly between islands for sites where the warbler was present, suggesting the warbler does not select structurally different sites on any island. Furthermore, habitat suitability did not predict warbler presence for any island, suggesting that other factors might play a role in Cape Verde warbler habitat selection at finer scales.

Unlike other local scale features, some plant species seem to predict warbler presence. The best model set indicated that reed, coffee, sugarcane and eucalypt are all significantly associated with warbler presence. Importantly the best model explained warbler presence considerably better than the null model. On S. Nicolau, the warbler seems to prefer plots with reed, on Fogo plots with coffee, and on Santiago plots with eucalyptus and sugarcane (Figure

4.7). Apart from *Eucalyptus* spp., which is present in mountain forests where the warbler had not been described previously, all the species were known from the existing anecdotal literature (Donald *et al.* 2004; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Fuchs 2009). For example, on Santiago the bird had been described in “plantations”, a term which in Cape Verde often includes sugarcane (Hazevoet 1995). On Fogo, it had been clearly associated with coffee plantations (Hering & Fuchs 2009; Hering & Hering 2005). On S. Nicolau it had only been found on reed stands, apart from occasional territories on dry river valleys holding both mango trees and patches of reed (Donald *et al.* 2004; Hazevoet *et al.* 1999). A degree of association between the warbler and certain plant species is therefore apparent, but the reason for this is unknown. These associations could be unrelated to a specific dependence of the warbler on certain plant species, but could reflect other factors, such as insect availability, suitability for nest structures, water availability (retention by the soil or irrigation of the fields, or moist brought by the clouds into the mountain forests), or other niche dimension or associated factor that was not directly measured in this generalist warbler.

None of the most abundant 14 plant species sampled in the plots (Table 4.4) are native to Cape Verde (Arechavaleta *et al.* 2005; Gomes *et al.* 2003). I found either plants used for agriculture, such as coffee, mango trees or sugarcane; trees used for forestry such as eucalypts, jacaranda *Jacaranda mimosifolia* and other trees of the Mimosaceae family; or exotic invasives such as Spanish-flag *Lantana camara*, reed, mesquite *Prosopis juliflora* and bushmint *Hyptis pectinata*. This indicates that most of the densely vegetated areas in Cape Verde are not covered in natural vegetation but are either agricultural or abandoned agriculture terrain. This makes it difficult to implement conservation actions since these areas are normally privately owned, rather than being located within protected areas. Plantations can be protected as long as there are incentives for agriculture. Conversely, the invasive plants are often subject to eradication programmes especially in the Natural Parks of Serra Malagueta, Monte Gordo and Fogo. The native scrub plant tortolho *Euphorbia tuckeyana*, previously hypothesised as the original Cape Verde warbler habitat (Hazevoet 1993; Hazevoet 1995), was present in only eight sampled plots, three with and five without the bird (data not shown). Taken together, these results suggest that while protecting native plant species has an intrinsic value for the preservation of the Cape Verdean biodiversity, it is unlikely to benefit the warbler, and removal of exotic vegetation used by the warbler can actually be harmful for this species.

Year-round dense green vegetation does not explain the habitat occupancy pattern of the Cape Verde warbler on S. Nicolau as it does on the other two islands (Table 4.3). In fact, on S. Nicolau, the importance of dry season NDVI for the habitat suitability maps was lower than on other islands (Table 4.3), which suggests that on this island evergreen dense vegetation cover predicts habitat suitability for the warbler less well than on other islands. On S. Nicolau the warbler was never found in the mountain forests, the lower sugar cane plantations or the *Prosopis* plantations (Figure 4.4), despite these sites being surveyed three times (in 2013 and 2014 during the breeding season, and in 2016 during the dry season, data not shown). Additionally, there are some differences in plant composition between islands when the sites where the warbler was present are compared (Figure 4.5). Importantly, it seems that the birds on S. Nicolau use a narrower range of plant species than the birds on Santiago and Fogo, but I did not find associations with specific structural traits. Overall, it seems that on Santiago and Fogo the warbler prefers agricultural or forested areas, while on S. Nicolau it is absent from such places and mostly confined to reed patches on mountain slopes. These results are in accordance with previous observations (Donald *et al.* 2004; Hazevoet 1993; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Fuchs 2009; Hering & Hering 2005). It appears that, on S. Nicolau, the warbler might not be using the entire suitable habitat available, indicating a non-saturated population. The reason for this is unclear. One possibility is that this population may be limited due to the detrimental effects of inbreeding depression (Brook *et al.* 2002; Crnokrak & Roff 1999; Hedrick & Kalinowski 2000). Genetic diversity in the S. Nicolau population is significantly lower than that of the other two populations (Batalha *et al.* 2017). Together with the limited number of territories found on the island (Batalha *et al.* 2017) and the fact that they do not seem to use all available habitat this could indicate the warblers on this island are on an extinction vortex (Frankham 2005; Frankham *et al.* 2009; Palomares *et al.* 2012), such as the Hawaiian crow *Corvus hawaiiensis* (Fagan & Holmes 2006). Alternatively, the warbler might not be using all available habitat because of the structural arrangement of vegetation on the different islands. For example, the coffee plantations on Fogo are kept in a continuous canopy layer, where the warblers hop from branch to branch; on S. Nicolau, farmers plant coffee interspersed with other crops and leave a large amount of open space in between them, which might make the warbler avoid it altogether (pers. obs.). In fact, the only coffee plantation where the warbler was present on S. Nicolau was a very small back yard where the coffee trees formed a closed canopy (Figure 4.8; pers. obs.). Other potential causes could be linked to stronger pathogen, predator or competitor prevalence in certain areas/habitats within that island (Padilla *et al.* 2017; Ruffino *et al.* 2015), but this needs further investigation. For example, climatic and anthropogenic factors affected haemosporidian parasite prevalence across habitats in an oceanic island (Padilla *et al.* 2017). It is currently unknown if the areas not used by the warbler on S. Nicolau, i.e. the mountain forest and the agricultural plantations, are more susceptible to diseases or predators or harbour more competitors than the abandoned reed stands and the valleys with mango trees.

Figure 4.9 Left: open space coffee *Coffea arabica* trees on S. Nicolau; right: Cape Verde warbler on a closed canopy coffee plantation on S. Nicolau, much like the ones present on Fogo. *Photos taken in April 2016.*

Implications for conservation

At a landscape level, the Cape Verde warbler inhabits areas which are covered in dense, evergreen vegetation. Green vegetation is obviously dependent on water availability, and prolonged droughts could potentially reduce the area available for the warbler on all the islands. The mountains in Cape Verde retain mist brought by the North-eastern trade winds, and for this reason areas on the North-eastern side of the islands can retain adequate levels of vegetation. Irrigation of agricultural fields and the recent building of dams also contribute to provide water in other areas of the islands. Therefore, to some extent, part of the warbler’s habitat, which could otherwise be threatened by climatic events such as droughts, could be naturally preserved by the mountains or by promoting suitable agricultural practices. Some plant species seemed to be favoured by the warbler, i.e. sugarcane plantations on Santiago, coffee plantations on Fogo, mountain forests on the previous two islands and reed stands on S. Nicolau. Thus different management practices may be needed on the three islands. Sugarcane and coffee plantations are agricultural crops, and ensuring that small scale farming remains on Santiago and Fogo should be sufficient to preserve a large part of the warbler’s habitat. Much of the remaining habitat on these two islands is forested, either drier *Prosopis* plantations at low level or mixed forests on mountains. However, on Fogo, I was unable to search for warblers throughout the inaccessible part of the forest of Monte Velha. It is important to verify if the warbler is present in the rest of the forested area of Fogo, or confined to the areas close to the coffee plantations. However, on S. Nicolau, the situation is different. The area with most warblers is in the large valley on the central part of the island, where the warbler is mainly found in large dense stands of reed, often on private agricultural land. I found some territories in the main dry river valleys where the habitat is mostly composed of mango trees and/or reeds and sugarcane. I did not find any evidence of presence of the Cape Verde warbler in many apparently suitable locations e.g. in the mountain forest of Monte Gordo, on the agricultural land on the main central valley or on the reed stands on the dry, southwestern part of Monte Gordo in either 2013, 2014 or 2016. The reason that the warbler is not present in many apparently suitable sites remains a mystery. Nevertheless, the fact that most of the territories on S. Nicolau are in reed stands suggests a different management strategy is required for this island. Reed is an invasive plant and the authorities encourage land owners to remove it, despite the fact that the warblers use it. The authorities currently have no legal way to ensure protection of warbler territories. The low genetic diversity of population of S. Nicolau (Batalha *et al.* 2017), combined with the well-intentioned but detrimental removal of the reeds which it uses to breed in, could have pushed this population to the verge of extinction. Finally, most of the territories found on Fogo and S. Nicolau in 2014 were outside the limits of the Natural Parks. This means that the authorities have limited power to assess and manage other threats to the Cape Verde warbler, such as diseases, predation, nest destruction or hunting. While this might not be important on Santiago, where suitable habitat is much more wide spread than on the other two islands, it is a problem on Fogo and S. Nicolau. I encourage the authorities on the latter two islands to find alternative ways to protect this bird's habitat, at the risk of these small, but genetically unique local warbler populations becoming extinct.

Conclusion

The Cape Verde warbler can be found on evergreen densely vegetated areas on all three islands where it exists. Evergreen vegetation, i.e. vegetation that stays green all year round, can comprise several plant species, from exotic shrubs to forest trees and irrigated agricultural plantations. However, the warbler seems to be associated with different species on the different islands. On Santiago, it can often be found on sugarcane plantations; on Fogo, most territories known to date are located in coffee plantations; on S. Nicolau, it is almost exclusively associated with large stands of exotic reed. The extent of evergreen vegetation areas differ between islands. On Santiago, it covers most of the island; on Fogo it is confined to the northeastern part of the island; on S. Nicolau it is limited to the central part of the island. However, on S. Nicolau, the warbler does not use the entire available potentially suitable habitat, being confined to the main valley on the central part of the island. Apart from evergreen dense vegetation and specific plant species, no structural habitat factors predicted the presence of the warbler on any of the three islands. This study is the first to assess in detail the habitat used by the warbler on all three islands and provides important information that can be used by practitioners to help define conservation measures tailored for each island. I suggest that associations of this bird with agricultural land and exotic plants are taken into account when planning for long-term conservation.

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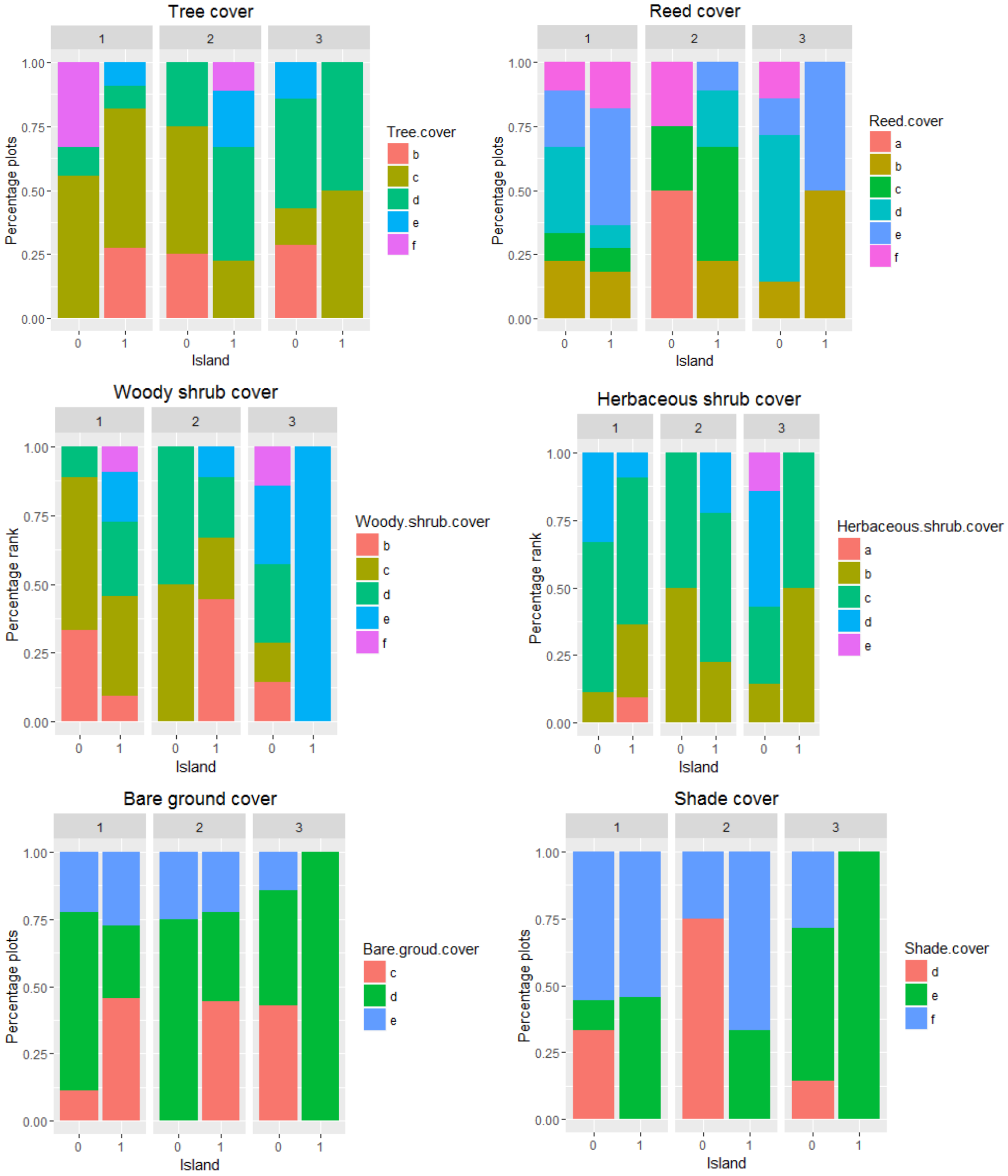
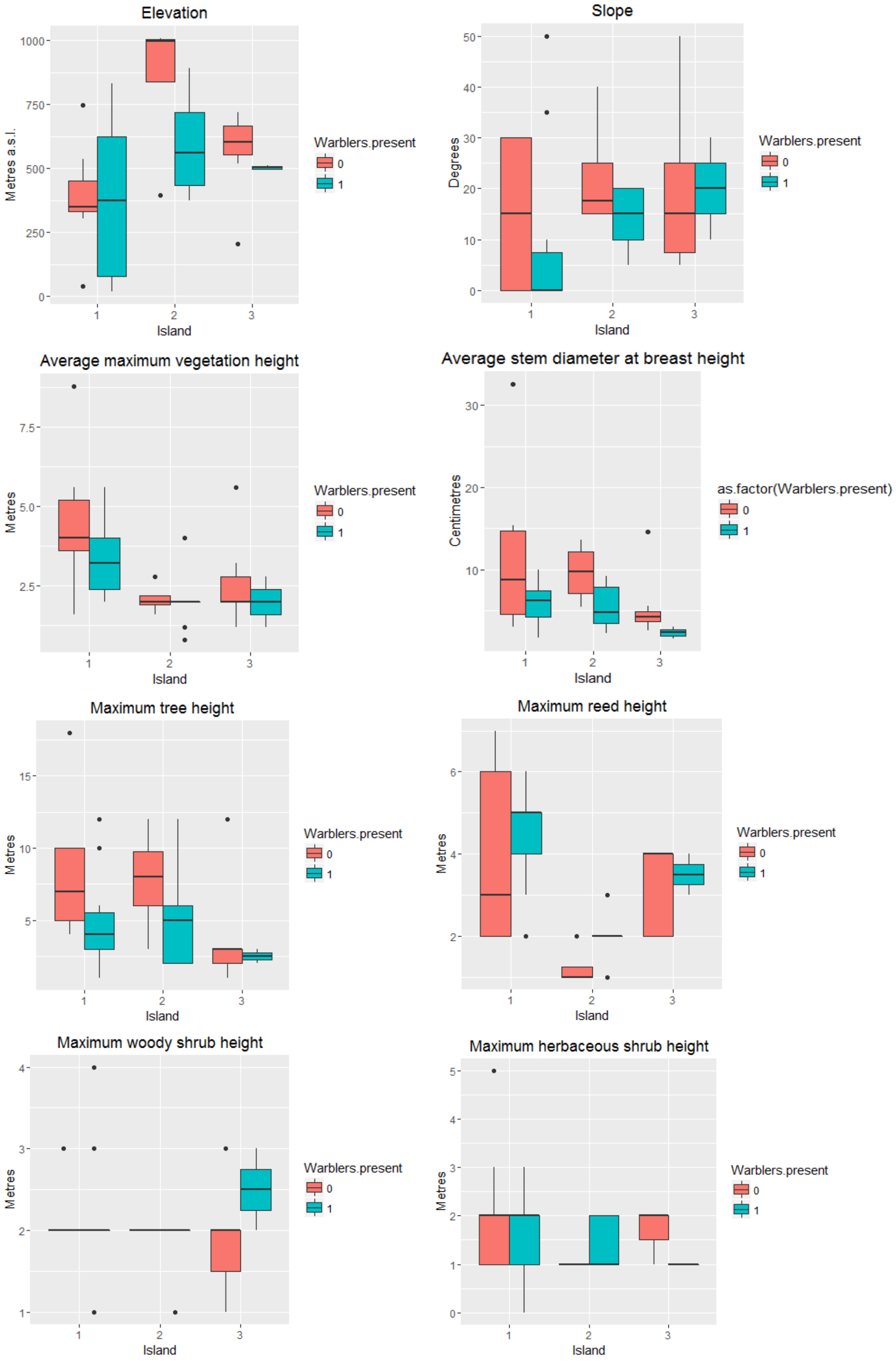
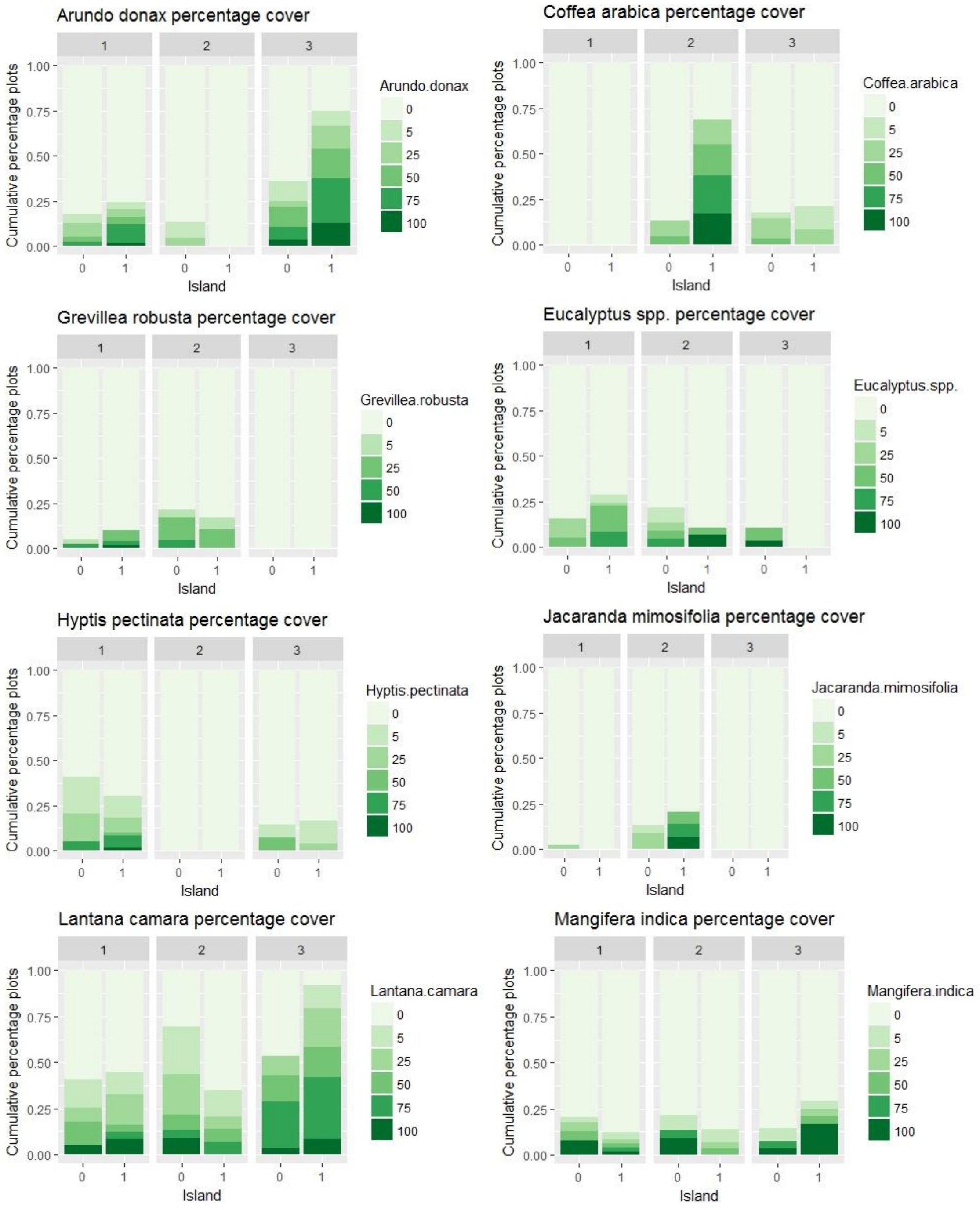
Supplementary figure S4.1 Barplots of percentage cover variables measured on the habitat sampling plots. Islands: 1 = Santiago; 2= Fogo; 3 = S. Nicolau. Within each island, plots where the Cape Verde warbler was 0 = absent and 1 = present.



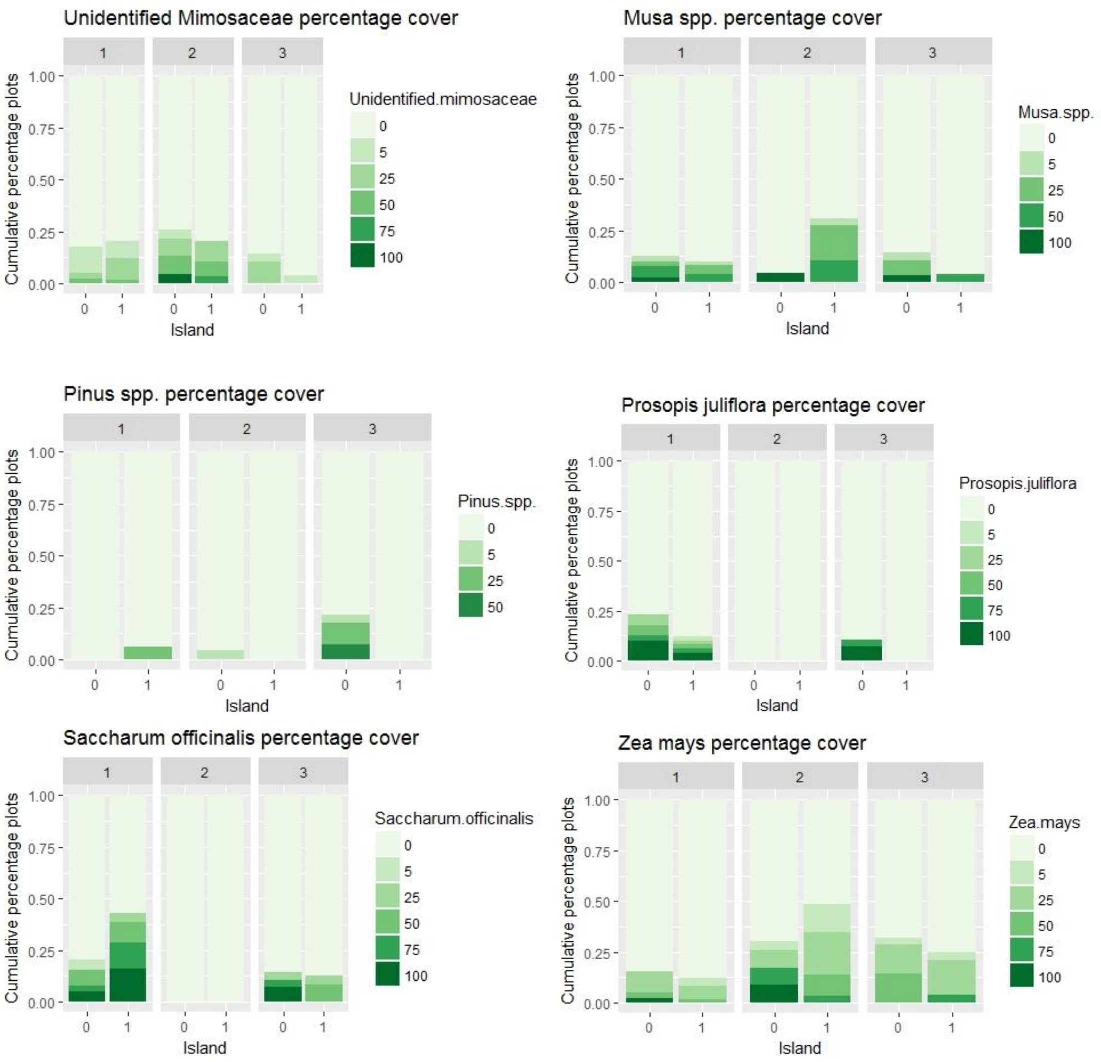
Supplementary figure S4.2 Boxplots of elevation, slope, diameter and height variables measured on the habitat sampling plots. Islands: 1 = Santiago; 2= Fogo; 3 = S. Nicolau. Within each island, plots where the Cape Verde warbler was 0 = absent and 1 = present.



Supplementary figure S4.3 Barplots of percentage cover of each species, genus or family of plants measured within the habitat sampling plots. Islands: 1 = Santiago; 2= Fogo; 3 = S. Nicolau. Within each island, plots where the Cape Verde warbler was 0 = absent and 1 = present.



Supplementary figure S4.3 (Cont.)



Supplementary table S4.1 Coefficients of logistic generalized linear mixed models (with ΔAIC ≤ 2 when compared to the best-fit model; n = 8) for Cape Verde warbler presence with coarse features, with degrees of freedom (df), log likelihood, AICc, delta AICc and model weight. All models include location as a random effect.

Intercept	Average maximum vegetation height	Bare ground cover	Elevation	Shade cover	Slope	df	logLik	AICc	delta	weight
0.587	0.155	-0.020				4	-109.559	227.3	0.00	0.153
-0.275	0.112					3	-111.367	228.9	1.53	0.071
0.190						2	-112.400	228.9	1.53	0.071
0.941		-0.014				3	-111.368	228.9	1.53	0.071
0.821	0.141	-0.019		-0.105		5	-109.312	228.9	1.61	0.068
0.667				-0.193		3	-111.412	229.0	1.62	0.068
1.068	0.162	-0.022	-0.001			5	-109.323	229.0	1.64	0.068
0.746	0.152	-0.020			-0.008	5	-109.462	229.2	1.91	0.059

Supplementary table S4.2 Coefficients of logistic generalized linear mixed models (with ΔAIC ≤ 2 when compared to the best-fit model; n = 22) for Cape Verde warbler presence with fine features with degrees of freedom (df), log likelihood, AICc, delta AICc and model weight. All models include location as a random effect.

Intercept	Average diameter	Herbaceous shrub cover	Maximum reed height	Maximum tree height	Maximum woody shrub height	Reed cover	Tree cover	Woody shrub cover	df	logLik	AICc	delta	weight
-2.893			0.347		-0.580	0.024	0.030	0.029	7	-105.321	225.3	0.00	0.081
-3.119			0.334			0.020	0.028	0.019	6	-106.599	225.7	0.40	0.067
-2.848		-0.022	0.382			0.020	0.027	0.018	7	-105.627	225.9	0.61	0.060
-2.674		-0.020	0.388		-0.541	0.023	0.030	0.028	8	-104.576	225.9	0.69	0.058
-0.184		-0.024	0.369						4	-108.908	226.0	0.78	0.055
-0.978		-0.023	0.467				0.012		5	-107.900	226.1	0.87	0.053
-2.365					-0.612	0.033	0.028	0.029	6	-106.846	226.1	0.90	0.052
-1.221			0.418				0.013		4	-109.090	226.4	1.14	0.046
-1.076			0.386	0.076					4	-109.093	226.4	1.15	0.046
-1.776		-0.023	0.517				0.017	0.013	6	-107.015	226.5	1.23	0.044
-0.409			0.315						3	-110.183	226.5	1.24	0.044
-2.041			0.471				0.017	0.013	5	-108.109	226.5	1.29	0.043
-0.775		-0.021	0.421	0.065					5	-108.153	226.6	1.38	0.041
-2.594						0.029	0.025	0.018	5	-108.158	226.6	1.39	0.041
-3.073			0.360	0.054	-0.572	0.024	0.026	0.029	8	-105.003	226.8	1.54	0.038
-2.675	-0.030		0.334		-0.621	0.025	0.034	0.028	8	-105.037	226.9	1.61	0.036
-1.427		-0.023	0.366			0.013	0.017		6	-107.236	226.9	1.68	0.035
-1.683			0.314			0.013	0.018		5	-108.345	227.0	1.76	0.034
-3.312			0.347	0.054		0.020	0.023	0.019	7	-106.241	227.1	1.84	0.032
-1.268			0.414		-0.474		0.015	0.018	6	-107.321	227.1	1.85	0.032
-1.642			0.413	0.092				0.011	5	-108.415	227.2	1.90	0.031
-1.470		-0.021	0.539		-0.422		0.018	0.019	7	-106.309	227.2	1.98	0.030

Supplementary table S4.3 Coefficients of logistic generalized linear mixed models (with ΔAIC ≤ 2 when compared to the best-fit model; n = 22) for Cape Verde warbler presence with plant species with degrees of freedom (df), log likelihood, AICc, delta AICc and model weight. All models include location as a random effect.

Intercept	*Arundo donax*	*Coffea arabica*	*Eucalyptus* spp.	*Grevillea robusta*	*Hyptis pectinata*	*Jacaranda mimosifolia*	*Mangifera indica*	*Musa* spp.	*Pinus* spp.	*Saccharum officinalis*	Unidentified Mimosaceae	*Zea mays*	df	logLik	AICc	delta	weight
-1.855	0.057	0.080	0.043			0.060				0.027			7.0	-95.720	206.0	0.00	0.077
-1.602	0.056	0.084	0.040			0.057				0.026		-0.023	8.0	-94.824	206.4	0.39	0.064
-1.994	0.057	0.082	0.038		0.024	0.062				0.029			8.0	-94.876	206.5	0.49	0.060
-1.999	0.059	0.083	0.042	0.031		0.060				0.028			8.0	-94.947	206.7	0.63	0.056
-2.143	0.060	0.085	0.046			0.063	0.014			0.030			8.0	-95.107	207.0	0.95	0.048
-1.714	0.056	0.079	0.046							0.026			6.0	-97.353	207.2	1.11	0.044
-1.743	0.059	0.087	0.040	0.030		0.057				0.027		-0.023	9.0	-94.102	207.2	1.15	0.044
-1.751	0.057	0.086	0.036		0.022	0.059				0.027		-0.021	9.0	-94.129	207.2	1.20	0.042
-1.448	0.056	0.083	0.043							0.025		-0.025	7.0	-96.321	207.3	1.20	0.042
-2.318	0.060	0.087	0.042		0.026	0.065	0.015			0.032			9.0	-94.137	207.3	1.21	0.042
-1.757	0.056	0.078	0.044			0.075				0.027	-0.027		8.0	-95.269	207.3	1.28	0.041
-2.139	0.060	0.085	0.039	0.029	0.024	0.062				0.030			9.0	-94.170	207.3	1.28	0.041
-1.785	0.055	0.078	0.049			0.058			-0.080	0.027			8.0	-95.271	207.3	1.28	0.041
-2.330	0.063	0.089	0.047	0.033		0.063	0.015			0.032			9.0	-94.233	207.5	1.41	0.038
-1.486	0.056	0.082	0.041			0.073				0.025	-0.029	-0.024	9.0	-94.280	207.5	1.50	0.036
-1.853	0.059	0.081	0.046	0.032						0.027			7.0	-96.530	207.7	1.62	0.034
-1.534	0.055	0.082	0.046			0.054		-0.083		0.026		-0.023	9.0	-94.354	207.7	1.65	0.034
-1.872	0.059	0.088	0.043			0.060	0.012			0.028		-0.021	9.0	-94.382	207.8	1.71	0.033
-1.843	0.057	0.081	0.042		0.023					0.027			7.0	-96.612	207.8	1.78	0.032

Supplementary table S4.3 (Cont.)

Intercept	*Arundo donax*	*Coffea arabica*	*Eucalyptus spp.*	*Grevillea robusta*	*Hyptis pectinata*	*Jacaranda mimosifolia*	*Mangifera indica*	*Musa spp.*	*Pinus spp.*	*Saccharum officinalis*	Unidentified Mimosaceae	*Zea mays*	df	logLik	AICc	delta	weight
-2.508	0.064	0.091	0.043	0.031	0.026	0.065	0.017			0.033			10.0	-93.329	207.9	1.83	0.031
-1.585	0.058	0.086	0.042	0.031						0.026		-0.025	8.0	-95.553	207.9	1.85	0.031
-1.783	0.055	0.081	0.042			0.060		-0.010		0.027			8.0	-95.570	207.9	1.88	0.030
-1.899	0.056	0.081	0.039		0.023	0.075				0.028	-0.024		9.0	-94.497	208.0	1.94	0.029
-1.922	0.056	0.081	0.043		0.023	0.060			-0.069	0.028			9.0	-94.526	208.0	1.99	0.029

Supplementary table S4.4 PCA on coarse habitat features, with standard deviation, proportion of variance, cumulative proportion of variance and loadings of each variable on each principal component, using only plots where warblers were present (n = 102).

	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.262	1.191	0.967	0.877	0.532
Proportion of Variance	0.318	0.284	0.187	0.154	0.057
Cumulative Proportion	0.318	0.602	0.789	0.943	1.000
Elevation	-0.495	0.442	-0.406	-0.368	0.508
Slope	-0.071	0.615	-0.189	0.728	-0.227
Bare.groud.cover	-0.333	-0.592	-0.189	0.555	0.441
Shade.cover	0.361	-0.199	-0.872	-0.099	-0.244
Average.maximum.vegetation.height	-0.713	-0.192	-0.052	-0.126	-0.660

Supplementary table S4.5 PCA on fine habitat features, with standard deviation, proportion of variance, cumulative proportion of variance and loadings of each variable on each principal component, using only plots where warblers were present (n = 102).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	1.868	1.318	1.173	0.850	0.741	0.605	0.548	0.524	0.432
Proportion of Variance	0.388	0.193	0.153	0.080	0.061	0.041	0.033	0.030	0.021
Cumulative Proportion	0.388	0.581	0.733	0.814	0.875	0.915	0.949	0.979	1.000
Tree.cover	0.445	-0.117	0.255	-0.050	0.306	-0.286	-0.213	0.225	0.670
Reed.cover	-0.399	-0.217	-0.350	0.274	0.105	0.112	0.280	-0.373	0.595
Woody.shrub.cover	-0.091	0.617	0.149	0.152	-0.653	-0.125	-0.014	0.037	0.351
Herbaceous.shrub.cover	-0.240	-0.315	0.570	0.069	-0.145	0.570	0.127	0.370	0.118
Maximum.tree.height	0.436	-0.048	0.078	0.442	-0.041	-0.171	0.740	0.079	-0.142
Maximum.reed.height	-0.416	-0.156	-0.210	0.386	0.023	-0.437	-0.158	0.619	-0.097
Maximum.herbaceous.shrub.height	-0.259	-0.322	0.545	-0.018	-0.132	-0.539	-0.020	-0.458	-0.115
Maximum.woody.shrub.height	-0.150	0.482	0.328	0.482	0.576	0.122	-0.147	-0.159	-0.109
Average.diameter	0.350	-0.311	-0.098	0.563	-0.312	0.197	-0.515	-0.219	-0.072

Supplementary table S4.6 PCA on plant species with standard deviation, proportion of variance, cumulative proportion of variance and loadings of each variable, using plots where warblers were *present and absent* (n = 192).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Standard deviation	1.527	1.269	1.160	1.131	1.087	1.070	1.003	0.962	0.876	0.855	0.743	0.675	0.645	0.502
Proportion of Variance	0.167	0.115	0.096	0.091	0.084	0.082	0.072	0.066	0.055	0.052	0.039	0.033	0.030	0.018
Cumulative Proportion	0.167	0.282	0.378	0.469	0.553	0.635	0.707	0.773	0.828	0.880	0.920	0.952	0.982	1.000
Arundo.donax	-0.003	0.497	-0.083	0.486	0.098	0.073	0.236	0.114	0.074	0.234	0.368	0.123	0.390	-0.252
Coffea.arabica	0.203	0.135	0.524	-0.285	-0.065	-0.014	-0.252	0.164	-0.478	0.368	-0.005	-0.014	0.077	-0.346
Eucalyptus.spp.	-0.455	-0.087	0.003	-0.284	-0.165	-0.132	-0.051	-0.099	0.091	0.002	0.729	-0.117	-0.220	-0.215
Grevillea.robusta	-0.268	-0.088	0.025	-0.280	0.321	0.390	-0.106	-0.129	0.485	0.492	-0.208	-0.017	0.171	-0.089
Hyptis.pectinata	-0.226	0.157	-0.281	-0.397	0.159	0.383	0.074	0.023	-0.385	-0.465	-0.041	0.126	0.322	-0.154
Jacaranda.mimosifolia	-0.335	-0.297	0.386	0.280	0.196	0.018	-0.026	0.069	-0.044	-0.147	-0.024	0.695	-0.108	-0.093
Lantana.camara	-0.426	0.359	-0.109	0.163	-0.124	0.045	0.061	0.210	-0.056	0.043	-0.419	-0.174	-0.528	-0.311
Mangifera.indica	0.128	-0.211	-0.315	0.229	-0.171	0.125	-0.716	0.244	0.152	-0.137	0.026	0.001	0.106	-0.341
Musa.spp.	0.185	-0.048	0.312	-0.206	-0.273	0.178	0.352	0.524	0.457	-0.287	0.002	-0.034	0.048	-0.156
Pinus.spp.	-0.261	0.093	-0.109	-0.183	-0.525	-0.478	-0.008	-0.076	0.117	0.104	-0.283	0.298	0.417	-0.008
Prosopis.juliflora	0.129	-0.154	-0.182	-0.143	0.549	-0.585	0.155	0.130	0.082	-0.063	-0.091	-0.060	0.017	-0.446
Saccharum.officinalis	0.200	-0.414	-0.161	0.111	-0.310	0.231	0.399	-0.406	-0.140	0.148	-0.067	0.049	-0.053	-0.475
Zea.mays	0.130	0.367	0.345	0.027	0.032	-0.035	-0.190	-0.603	0.281	-0.412	-0.084	-0.047	-0.017	-0.261
Unidentified.mimosaceae	-0.378	-0.304	0.302	0.314	0.013	-0.051	0.043	0.001	-0.133	-0.141	-0.108	-0.585	0.423	-0.018

Supplementary table S4.7 PCA on plant species with standard deviation, proportion of variance, cumulative proportion of variance and loadings of each variable, using only plots where warblers were present (n = 102).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Standard deviation	1.555	1.313	1.246	1.239	1.064	1.038	1.012	0.930	0.821	0.803	0.702	0.633	0.564	0.374
Proportion of Variance	0.173	0.123	0.111	0.110	0.081	0.077	0.073	0.062	0.048	0.046	0.035	0.029	0.023	0.010
Cumulative Proportion	0.173	0.296	0.407	0.517	0.597	0.674	0.747	0.809	0.857	0.903	0.939	0.967	0.990	1.000
Arundo.donax	0.028	-0.407	-0.235	0.483	0.082	0.003	0.220	0.053	0.297	0.087	0.432	0.299	0.041	-0.337
Coffea.arabica	0.311	-0.096	0.521	-0.171	-0.054	0.000	-0.143	-0.026	0.358	-0.442	-0.110	0.111	-0.172	-0.439
Eucalyptus.spp.	-0.459	-0.062	0.110	-0.323	-0.145	0.116	0.005	-0.131	0.044	0.072	0.522	-0.497	-0.111	-0.281
Grevillea.robusta	-0.288	0.050	0.155	-0.183	0.469	-0.390	0.020	-0.029	0.352	0.507	-0.240	0.133	-0.131	-0.100
Hyptis.pectinata	-0.279	-0.227	-0.064	-0.367	0.260	-0.270	0.155	0.099	-0.458	-0.421	0.098	0.375	0.109	-0.093
Jacaranda.mimosifolia	-0.359	0.279	0.350	0.333	-0.009	0.051	-0.162	0.018	-0.037	-0.042	-0.053	0.063	0.691	-0.207
Lantana.camara	-0.323	-0.396	-0.112	0.272	-0.169	-0.054	-0.076	0.306	-0.135	-0.067	-0.547	-0.304	-0.208	-0.254
Mangifera.indica	0.073	0.122	-0.317	0.005	-0.193	-0.360	-0.735	-0.148	-0.144	0.101	0.137	0.160	-0.082	-0.261
Musa.spp.	0.238	-0.073	0.370	-0.098	-0.208	-0.097	-0.005	0.663	-0.269	0.416	0.216	0.073	0.011	-0.027
Pinus.spp.	-0.233	-0.193	-0.063	-0.327	-0.505	0.380	0.004	-0.135	0.135	0.237	-0.211	0.505	0.080	0.019
Prosopis.juliflora	0.057	0.090	-0.131	-0.079	0.520	0.679	-0.261	0.169	-0.180	0.105	-0.039	0.058	-0.107	-0.279
Saccharum.officinalis	0.134	0.520	-0.265	-0.084	-0.194	-0.081	0.500	0.020	-0.109	0.064	-0.151	0.001	-0.023	-0.548
Zea.mays	0.214	-0.290	0.310	0.139	0.050	0.004	0.120	-0.604	-0.496	0.298	-0.093	-0.037	-0.013	-0.166
Unidentified.mimosaceae	-0.339	0.334	0.271	0.362	-0.087	0.071	0.043	-0.027	-0.162	-0.092	0.131	0.326	-0.617	0.118

Chapter 5

Morphological variation in the Cape Verde warbler *Acrocephalus brevipennis*

Colour-ringed Cape Verde warbler Acrocephalus brevipennis *photographed in a reed* Arundo donax *stand in Canto Fajã, S. Nicolau, September 2016.*

5.1 Abstract

Oceanic islands are excellent systems to study speciation because the isolated populations they contain will diverge over time in the absence of gene flow. Morphological divergence can be caused by neutral forces or be linked to selection by environmental factors. Quantifying morphological divergence can help disentangle the role of environmental forces in driving population divergence. Here I use morphological traits to quantify the degree of divergence between the three extant populations of the endangered Cape Verde warbler *Acrocephalus brevipennis*. Results show some morphological divergence between the smallest and most isolated population of S. Nicolau and the more closely related populations of Santiago and Fogo, when controlling for sexual dimorphism. This divergence is not related to overall body size, but specifically to tarsus length and bill length and shape. Birds from S. Nicolau have shorter tarsi and longer, more pointed bills than those from Santiago and Fogo. These morphological differences most likely reflect the evolutionary history and colonisation patterns of the Cape Verde warbler, because the population on S. Nicolau was isolated from the other two populations earlier than Fogo was isolated from Santiago. The S. Nicolau population has also been through a recent bottleneck. However, this analysis cannot exclude the possibility that these morphological differences could be driven by rapid adaptation to different environments across the islands, or by a combination of drift and adaptation. Regardless of the forces driving this morphological variation, my findings are consistent with what would be expected in the initial stages of allopatric speciation.

5.2 Introduction

Oceanic islands provide excellent model systems to study population divergence and speciation (MacArthur & Wilson 1967; Whittaker 1998; Wright 1931). They constitute discrete geographical entities between which gene flow is often limited or non-existent (Cibois *et al.* 2011; Warren *et al.* 2014; Whittaker 1998) and are therefore a suitable setting for studying how different environmental conditions drive divergence in isolated populations (Grant 1986; Warren *et al.* 2014; Whittaker 1998). Different environmental conditions might be present on each island, or within different habitats on single islands, promoting ecological divergence of populations over relatively small areas (Grant 1986; McKinnon *et al.* 2004; Schluter 2000; Schluter 2001). Furthermore, islands are simplified ecosystems when compared to mainland areas (MacArthur & Wilson 1967; Traveset *et al.* 2016; Warren *et al.* 2014; Whittaker 1998). This can make the study of speciation in such systems more straightforward when compared with mainland systems (Grant 1986; McKinnon *et al.* 2004; Schluter 2000; Schluter 2001). Selective forces can be strong on islands, because populations must survive in new ecological niches and environmental conditions (Grant 1985; Grant 1986). Conversely, colonisers often undergo an ecological release, also called “enemy” release, whereby during island colonisation they are released from the predators, parasites and/or competitors they faced on the mainland (Bolnick *et al.* 2010; Ghazoul 2002; Keane & Crawley 2002; Sih *et al.* 2010). This allows for changes in their ecological niche and morphology that would otherwise be constrained by their “enemies”. All the above reasons explain why island systems have been central to understanding many facets of evolution and ecology (Grant 1998; Warren *et al.* 2014).

Speciation is a gradual, time-dependent process which is concluded when there is complete reproductive isolation between two populations (Dobzhansky 1937; Mayr 1942). This develops over three stages: two populations become geographically isolated, one or both of the populations diverge from how they were initially, and there is secondary contact but no interbreeding between the two now diverged populations (De Queiroz & Weins 2007; Orr & Smith 1998; Schluter 2001). When secondary contact does not occur in nature e.g. island populations, it is not possible to verify if reproductive isolation is complete (De Queiroz & Weins 2007; Orr & Smith 1998; Schluter 2001). It is therefore necessary to compare population traits such as genetics, morphology, habitat or behaviour and use them as a proxy to determine whether they are full species, e.g. by comparing them with known pairs of sister species (Avise & Wollenberg 1997; Helbig *et al.* 2002; Mayr 1942). Most natural populations are at a point in a time-dependent continuum of speciation, from homogeneous populations through to distinct species (Peccoud *et al.* 2009; Shaw & Mullen 2014; Supple *et al.* 2013). Extremes on this continuum are easily identified, but populations in the "grey area" of speciation are less easy to classify, and their status as discrete entities is often ambiguous (Coyne & Orr 2004; Orr & Smith 1998; Roux *et al.* 2016). When trying to understand what processes created the variation observed in a group of populations, it is useful to quantify the degree of divergence between them and determine at what point in the speciation continuum the taxa are. This also has implications for how we categorise and catalogue biodiversity and, in turn, how to effectively prioritise limited resources for the conservation of biodiversity (Moritz 1994; Ryder 1986).

Variation between populations or species can be genetic, ecological, behavioural and phenotypic, or a combination of these (Coyne & Orr 2004; Milá *et al.* 2007; Price 2008). Morphology, a term commonly used to refer to the size and shape of individuals, can vary for different populations of the same species, without necessarily correlating to subspecies (Grant 1965, 1979; Price 2008). Morphological traits can be easily measured and compared across populations to assess divergence (Ratciliffe & Grant 1983; Schluter & Grant 1984a) and have been one of the most commonly used set of traits in assessing and identifying species, or other units below the species level (Langerhans *et al.* 2003; Perera *et al.* 2007; Ratciliffe & Grant 1983; Vasconcelos *et al.* 2012). Morphological data often form a preliminary basis for further genetic study, or are used in conjunction with genetic studies to determine intraspecific taxon classification (Arnold *et al.* 2008; Foote *et al.* 2009; Gruber *et al.* 2013; Nicholls & Austin 2005).

The relative effects of neutral and adaptive forces in driving population divergence are often difficult to disentangle and quantify (Clegg *et al.* 2002b; Sutton *et al.* 2011; Westerdahl *et al.* 2004). Stochastic forces can shape morphological traits in the absence of or despite deterministic effects of selection (Rocamora & Richardson 2003; Slatkin 1987; Spurgin *et al.* 2014). Indeed, non-adaptive evolution is a significant evolutionary force (Allendorf & Luikart 2007; Hartl & Clark 1997; Kimura & Ota 1974; Wright 1969). Evolutionary differences between island populations can be the result of different colonisation histories (Emerson 2002; Hille *et al.* 2003; Illera *et al.* 2014) or other neutral forces such as drift, bottlenecks and founder effects at the genetic level (Kolbe *et al.* 2012; Miller & Lambert 2004; Ramstad *et al.* 2013). Many studies found support for the overriding role of drift in shaping divergence between populations in both genetic and morphological traits (Gonzalez-Quevedo *et al.* 2015; Grueber *et al.* 2013; Miller & Lambert 2004; Spurgin *et al.* 2014). In some cases discordance between morphological and genetic divergence may be caused by phenotypic plasticity in response to environmental differences, as opposed to selection (Gruber *et al.* 2013). In some cases, a combination of neutral and adaptive forces seems to have shaped divergence of populations and a combination of morphological and genetic research has been crucial in disentangling their impacts under various systems and scenarios (Clegg *et al.* 2002a; Clegg *et al.* 2002b; Clegg & Phillimore 2010; Potvin & Clegg 2015). Mismatches between genetic and phenotypic patterns can indicate action of selective forces, especially when phenotypic patterns match specific environmental factors (Funk *et al.* 2008; Langerhans *et al.* 2003; McKinney *et al.* 2014). In some instances, population divergence has been shown to reflect adaptations to local environments (Grant 1965, 1986; Schluter & Grant 1984a). Rapid evolution has been linked to selection, as in the classic case of the adaptive radiation of the Galapagos finches (Bowman 1961; Grant 1985; Grant & Grant 1993; Grant 1986; Grant 2008). Long term studies on the Galapagos finches have shown how natural selection driven by food resources acted on these population to drive morphological divergence, specifically bill shape and size (Grant *et al.* 1985; Grant & Grant 2004; Grant 2008). However, it is only possible to identify the action of selective forces with long term studies and/or examination of biologically relevant environmental factors (Bell 2010; Clegg *et al.* 2008; Grant 2008).

The Cape Verde warbler *Acrocephalus brevipennis* is an endangered passerine endemic to Cape Verde, a volcanic archipelago ca. 500 km off West Africa, between 14–18°N and 22–26°W in the Atlantic Ocean (Figure 5.1). It currently exists on three islands, Santiago, Fogo and S. Nicolau (Batalha *et al.* 2017). Fogo and Santiago, in the southern Sotavento group of islands, are separated by ca. 60 km, whereas S. Nicolau, in the northwestern Barlavento group, is separated from the former two islands by more than 160 km (Figure 5.1). Such oceanic barriers can prevent gene flow, especially between the Sotavento and Barlavento groups (Hazevoet 1995; Hille *et al.* 2003), which are differently affected by the northeastern trade winds (Correia 1996; Philander *et al.* 1996). Previous research indicates recent genetic divergence between the populations (Batalha *et al.* 2017), but whether morphological differences exist is unknown. No documented measurements of live birds exist apart from a few measurements taken on S. Nicolau when the bird was discovered 150 years ago (Dohrn 1871; Keulemans 1866); however, the sample size is too small to make any meaningful comparisons.

Here I investigate morphological differences between the three extant populations of the Cape Verde warbler, while assessing and controlling for sexual dimorphism. If neutral factors are the overriding force shaping morphological divergence, I expect any differences to align with the neutral genetic divergence patterns previously documented (Batalha *et al.* 2017). If adaptive forces are predominant, morphological and genetic divergence will exhibit discordant patterns (Clegg *et al.* 2002b). I also used this opportunity to record moult patterns of adult birds.

5.3 Methods

5.3.1 Data collection

Birds were sampled across Santiago, Fogo and S. Nicolau from November 2013 to January 2014, and from September to November 2014 during the main breeding season (Hazevoet 1995). I surveyed for warblers across all potential suitable habitat, i.e. densely vegetated areas (Cramp & Perrins 1992; Hazevoet 1995) and all altitudes on each island (Figure 5.1). On Santiago, dense vegetation can be found over the whole island including coastal plantations and mountain forests; on Fogo, vegetated areas are confined to the wetter, northeastern part of the island and a few sites on the northwestern side; on S. Nicolau, vegetated areas are located in the mountain valleys of the central area of the island (Figure 5.1). After confirming it was absent from more barren areas, I restricted my searches to areas with all kinds of vegetation, including areas where the bird had been previously detected, by me or previous observers (Batalha *et al.* 2017; Donald *et al.* 2004; Hazevoet 1995; Hazevoet *et al.* 1999; Hering & Fuchs 2009), and areas with dense vegetation but where the bird had not been recorded before. This means that the sampling was not completely random, thus artificially increasing the precision of finding the birds; however, the alternative would be to risk not obtaining a large enough sample size.

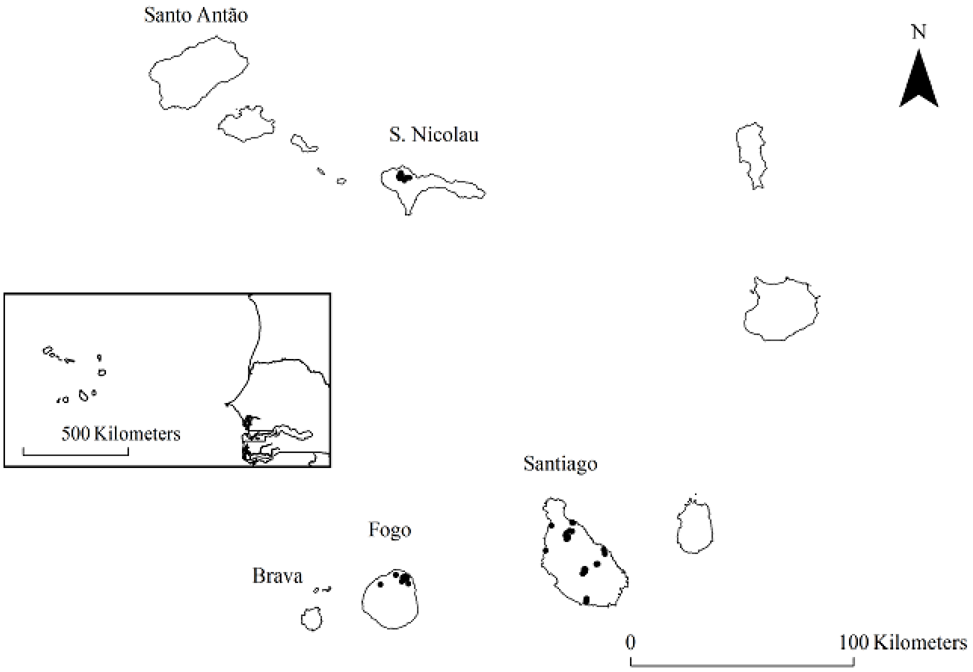


Figure 5.1 Map of the Cape Verde islands (main) with position relative to West Africa (inset), with all the locations where Cape Verde warblers were sampled in 2013 and 2014, on Santiago, Fogo and S. Nicolau. *Map produced using open source data on ArcMap 10.1 by Helena Batalha.*

Individuals were located by their song bursts while conducting transects in densely vegetated areas. The birds were then lured into mist nets with playback songs downloaded from Xeno-Canto.org (recordings numbers XC156923 to XC156925) or recorded by me in the field. Each caught bird was fitted with a unique combination of a numbered Cape Verde metal ring, and three UV-resistant plastic colour rings, and each sampling location was recorded with a Garmin eTrex® H GPS. All individuals were measured twice by the same person (H.R.B.) using a digital calliper (±0.01 mm), ruler (±1 mm) or digital scale (±0.01 g). Measurements were taken according to Svensson (1992), unless stated otherwise. The following measurements were taken: *wing length* (maximum length of the flattened and straightened wing); minimum *right tarsus length* (bent method, from the intertarsal joint to the other end of the bone (Redfern & Clark 2001)); *head + bill* (head length from posterior end of skull to tip of bill (Redfern & Clark 2001)); *bill length* (from bill tip to the anterior edge of nostril); *bill depth* (on a 90° angle relative to the bill’s horizontal plane at gape); *bill width* (at gape); *tail length* (from the base of the two central tail feathers to their tips, only taken when individuals were not moulting); and *weight*. Moult patterns were assessed using British Trust for Ornithology codes (Ginn & Melville 1983; Redfern & Clark 2001). The age of all birds was estimated in the hand: warbler plumage colour is more rufous in juveniles and more grey in adults (Cramp & Perrins 1992) and, as in other *Acrocephalus*, iris colour is grey in juveniles and brown in adults (Baker 1997; Cramp & Perrins 1992; Komdeur 1991). Tongue spots were not used to help determine bird age (Leisler & Schulze-Hagen 2011; Svensson 1992) because they were present in juveniles and adults. When neither plumage or iris colour could be reliably used to age the bird, it was classified as age unknown and excluded from the analyses, which included only adult birds. Blood samples (about 40 µl) were collected by brachial venipuncture, preserved in 800 µl of absolute ethanol in a screw-cap microfuge tube, and stored at room temperature. Birds were released in the same place where they were caught. Individual sex was confirmed molecularly as sexes are said to be monomorphic in the Cape Verde warbler (Cramp & Perrins 1992). DNA was extracted using a salt extraction protocol following Richardson *et al.* (2001), and individuals were sexed following Griffiths *et al.* (1998). I used P2 and P3 primers to amplify CHD genes (males, ZZ; females ZW). Polymerase chain reactions (PCRs) were performed in 10 µl total volume, consisting of 5 µl TopTaq Master Mix (Qiagen, West Sussex, UK), 1 µl (5 µM) each primer, 0.4 µl CoralLoad buffer, 2.6 µl ddH2O and 1 µl DNA (ca. 25ng/µl). Thermal cycle conditions were 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 45 s, with a final extension step at 72°C for 10 min and a cooling step at 20°C for 1 min. PCR products were visualised in 1.5% agarose gel, single bands being scored as males and double bands as females.

5.3.2 Statistical analyses

All statistical analyses were done in R (R Core Team 2016). I first assessed sexual dimorphism in adult birds. Only birds for which all the measurements had been taken (except tail and weight) were included in the analyses. Normality and homogeneity of variance of the data were assessed using Shapiro Wilks test with function shapiro.test, package stats (R Core Team 2016) and Levene tests with function leveneTest, package car (Fox & Weisberg 2011). For males, wing length, bill depth and bill width were not normally distributed (W = 0.951, 0.967 and 0.966, p = 0.004, 0.038 and 0.034, respectively). For females, bill width was not normally distributed (W = 0.891, p = 0.006). All other variables were normally distributed (all p > 0.054). Variances were not equal for tail (Levene's test 6.469, p = 0.0160). I then tested for differences between males and females in each trait with Kruskal-Wallis, Mann-Whitney or t-tests (functions kruskal.test, wilcox.test and t.test, package stats). Because many birds were moulting tail feathers, especially after November, tail measurements were only recorded for 35 adult birds. For this reason, tail length was not included in the multivariate analyses. I also tested for multicollinearity between morphological variables for the two sexes and for adult males only with the function vif, package car, and there was no multicollinearity in either dataset (all VIF between 1.401 - 2.826 for all birds and between 1.097 and 2.882 for adult males). Regardless, I tested for correlations between all the variables with function corr.test, package psych (Revelle 2014). In the dataset with all adult birds, head + bill was strongly and significantly correlated to bill to nostril and weight (Spearman's rho = 0.67 and 0.52 respectively, $p < 0.001$), and wing was strongly and significantly correlated to tail (Spearman's rho = 0.81, $p < 0.001$). For adult males, head + bill and bill to nostril, as well as wing and tail were strongly and significantly correlated (Spearman's rho = 0.69 and 0.76 respectively, $p < 0.001$).

Because of the correlation between morphological traits, and because several traits should be used to represent of bird size and shape (Freeman & Jackson 1990), it is useful to extract the principal components of variance and use these to test for differences between sexes or populations (James 1982). I performed a principal components analysis (PCA) to visualise differences between males and females (hereafter sex PCA), including all sampled individuals for which we had all measurements. Tail was not included in the analyses. The first component explained most of the variance and, when plotted, clearly discriminated between sexes (Table 5.3 and Figure 5.2). Therefore I tested for differences between sexes with an ANOVA with the first component (function aov, package stats).

I performed a second PCA to determine the occurrence of population structure based on the morphological traits (except tail length) across the three populations (hereafter island PCA). Because there were significant differences between males and females, and because most of the individuals measured were males, I only used adult males in this analysis. For this PCA, only the second component discriminated between the three islands (Table 5.4 and Figure 5.3). I tested for morphological differences between populations with an ANOVA using the first and the second components, followed by a post-hoc Tukey test for the ANOVA on the island PCA components (functions aov and TukeyHSD, package stats).

To test whether bill shape was different between islands, I created a bill shape index: Bill shape = Bill length to nostril / (bill depth * bill width). This index was created based on commonly used formulas for calculating bill shape indexes. In general, these indexes divide bill length by bill depth to obtain a measure of pointedness (Batalha *et al.* 2013; Jones 1993; Sutherland *et* *al.* 2004). Because I had also measured bill width, this index divides bill length by the product of bill depth and bill width. Higher values of this index indicate longer and narrower bills, and lower values indicate shorter and thicker bills. As it is not a univariate morphological trait but rather an index, the bill shape index was not included in the island PCA but examined separately. I tested for differences in bill shape between populations with an ANOVA on the bill shape index, followed by a post-hoc Tukey test.

5.4 Results

5.4.1 Morphological data

I recorded morphometric data of 138 birds, of which 113 were adults, 16 were juveniles and nine were of undetermined age. Molecular sexing revealed that 95 of these birds were males and 43 were females. For all the analyses, only birds for which I had all the measurements (except tail) were used. Averages and standard error of each morphological trait per sex and per island are given on Table 5.1.

Table 5.1 Morphological measurements of adult Cape Verde warblers per sex (in italic) and per island (separated for each sex). For each trait, average and standard error (SE) are given in millimetres. Note that I could not measure tail in all the examined birds, hence the sample size is smaller than for other traits. Traits were measured on live, wild living animals, from November 2013 to January 2014 and from September to November 2014. For more details see main text and Table S4.1.

		Wing		**Tarsus**		**Head + bill**		**Bill to nostril**	
	N	**Average**	**SE**	**Average**	**SE**	**Average**	**SE**	**Average**	**SE**
Males	*78*	*65.25*	*0.17*	*27.00*	*0.11*	*39.47*	*0.09*	*11.59*	*0.06*
Santiago	33	65.32	0.28	27.29	0.16	39.23	0.13	11.47	0.10
Fogo	27	65.44	0.32	27.25	0.17	39.45	0.13	11.39	0.07
S. Nicolau	18	64.83	0.28	26.11	0.18	39.93	0.16	12.12	0.09
Females	*29*	*62.66*	*0.26*	*25.65*	*0.15*	*38.21*	*0.13*	*11.19*	*0.09*
Santiago	17	62.85	0.37	25.82	0.18	38.02	0.15	11.21	0.11
Fogo	6	61.83	0.60	26.06	0.12	38.12	0.25	10.90	0.25
S. Nicolau	6	62.92	0.24	24.76	0.25	38.83	0.29	11.43	0.13

		Bill depth		**Bill width**		**Tail**			**Weight**	
	N	**Average**	**SE**	**Average**	**SE**	**Average**	**SE**	**N tail**	**Average**	**SE**
Males	*78*	*3.61*	*0.01*	*5.40*	*0.02*	*63.21*	*5.29*	*28*	*17.87*	*0.12*
Santiago	33	3.60	0.02	5.39	0.04	63.20	3.16	10	17.64	0.20
Fogo	27	3.64	0.03	5.44	0.03	64.60	3.16	10	17.86	0.17
S. Nicolau	18	3.61	0.03	5.38	0.04	61.50	2.83	8	18.28	0.29
Females	*29*	*3.53*	*0.03*	*5.28*	*0.03*	*60.14*	*2.65*	*7*	*16.63*	*0.20*
Santiago	17	3.56	0.04	5.31	0.03	60.67	1.73	3	16.64	0.26
Fogo	6	3.53	0.08	5.30	0.07	60.50	1.41	2	16.45	0.33
S. Nicolau	6	3.43	0.04	5.19	0.08	59.00	1.41	2	16.78	0.56

5.4.2 Sexual dimorphism

In all analyses, I used 107 birds for which I had measured all morphological traits except tail, in a total of 78 males and 29 females. Univariate tests showed differences between males and females for all morphological traits, with males being larger than females (Table 5.2).

Table 5.2 Sexual dimorphism in the Cape Verde warbler; non-normal variables were tested with a Mann-Whitney test, normal variables with a t-test, and variables with unequal variances with a Kruskal-Wallis test. All the variables differ significantly between males and females.

	N Males	N Females	test used	test value	df	p value
Wing	78	29	Mann-Whitney	228.50	NA	<0.001
Tarsus	78	29	t-test	-7.37	63.02	<0.001
Head + bill	78	29	t-test	-8.14	54.46	<0.001
Bill length	78	29	t-test	-3.73	55.98	<0.001
Bill depth	78	29	Mann-Whitney	773.50	NA	0.012
Bill width	78	29	Mann-Whitney	652.00	NA	<0.001
Tail	28	7	Kruskal-Wallis	8.64	1.00	0.003
Weight	78	29	t-test	-5.30	51.67	<0.001

Multivariate analysis confirmed the morphological differences between sexes. Individuals for which not all of the traits had been measured (e.g. they escaped while being measured) were not included in the sex PCA. The first and second components explained 45% and 15% of the variance, respectively (Table 5.3). The first principal component, related to overall size, appeared to explain variation between males and females, while the second component explained variation within each sex (Figure 5.2). Two traits, tarsus and bill length, contribute disproportionately to the second component, suggesting that these are the features that explain most variance within each sex (Table 5.3). Taken together, this indicates that males are larger than females overall and that no morphological traits contribute disproportionately to sexual dimorphism in this species (Table 5.3, Figure 5.2).

Table 5.3 Principal component analyses of sexual dimorphism in the Cape Verde warbler, using only adult birds; standard deviation, proportion of variance, cumulative proportion of variance of first and second principal components and loadings of each traits on each component are shown (n = 107).

		PC1	PC2
	Standard deviation	1.77	1.04
	Proportion of Variance	0.45	0.15
	Cumulative Proportion	0.49	0.60
Loadings	Wing	-0.36	-0.20
	Tarsus	-0.35	-0.59
	Head + bill	-0.47	0.31
	Bill length	-0.35	0.69
	Bill depth	-0.33	-0.15
	Bill width	-0.35	-0.07
	Weight	-0.42	-0.08

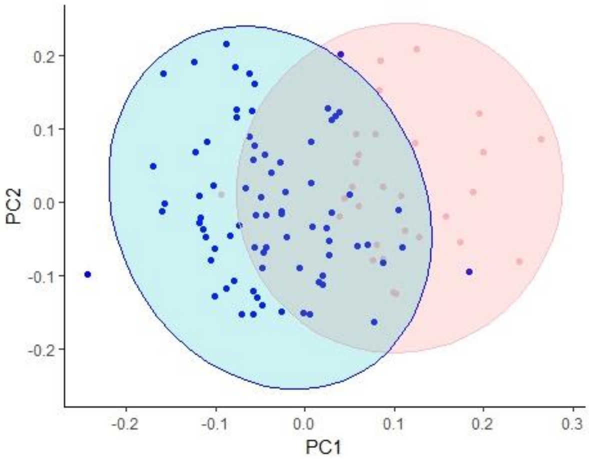


Figure 5.2 Principal components analysis of sexual dimorphism in the Cape Verde warbler; groups correspond to males (blue, n = 78) and females (pink, n = 42). Ellipses show 95% confidence intervals.

5.4.3 Differences between populations

For the island PCA, I used the 78 adult males for which all morphological traits were measured (Santiago, n = 33, Fogo, n = 27 and S. Nicolau, n = 18). The first and second components explained 33% and 18% of the variance, respectively (Table 5.4). In this case, the first component seemed to explain variance within each population and the second principal component seemed to explain variance between the S. Nicolau population and the two Sotavento populations, i.e. Santiago and Fogo (Figure 5.3). For the first component, head + bill, bill length and weight were the traits with highest loadings (Table 5.4). For the second component, tarsus and bill length were the traits with the highest loadings (Table 5.4). As the second component seems to explain variance between populations better than the first component (Figure 5.3), I inferred that tarsus and bill length are the traits that differ more between populations, particularly between S. Nicolau and the southern islands of Santiago and Fogo.

Table 5.4 Principal component analyses of island differences in the Cape Verde warbler; standard deviation, proportion of variance, cumulative proportion of variance of first and second principal components and loadings of each traits on each component are shown (n = 78).

		PC1	PC2
	Standard deviation	1.53	1.13
	Proportion of Variance	0.33	0.18
	Cumulative Proportion	0.33	0.52
Loadings	Wing	0.11	-0.29
	Tarsus	0.18	-0.68
	Head + bill	0.58	0.20
	Bill length	0.45	0.55
	Bill depth	0.32	-0.22
	Bill width	0.35	-0.15
	Weight	0.45	-0.19

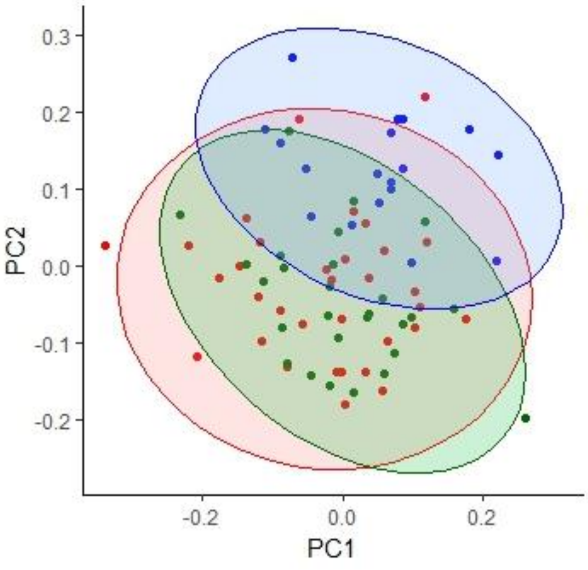


Figure 5.3 Principal components analysis of morphometric differences in the adult male Cape Verde warblers; groups correspond to the populations of Santiago (red, n = 33), Fogo (green, n = 27) and S. Nicolau (blue, n = 18). Ellipses show 95% confidence intervals.

There was no difference between islands for island PC1 (F = 3.121, p = 0.050), but there was for island PC2 (F = 26.140, p < 0.001). This indicates that the second component, rather than the first, explains morphological variance between islands. Post-hoc Tukey tests revealed that PC1 shows only significant differences between the populations of Santiago and S. Nicolau (Table 5.5). For PC2 there were significant differences between S. Nicolau and the other two populations, i.e. Santiago and Fogo, but not between Santiago and Fogo (Table 5.5). Accordingly, ANOVAs on the morphological traits that most contributed to PC2 showed the same results. Bill length (F = 13.8, p < 0.001), tarsus length (F = 11.76, p < 0.001) and bill shape, which was not included in the PCA (F = 12.79, p < 0.001), differed significantly between islands. Post-hoc Tukey tests supported the results for PC2 by revealing that bill length, tarsus length and bill shape are significantly different between S. Nicolau and the two Sotavento populations (Table 5.5).

Table 5.5 Post-hoc tests of morphological differences between adult males of the three populations of Cape Verde warbler. The table shows the results of Tukey tests following ANOVAs on the first and second components of the principal component analysis with all morphological traits measured (PC1 and PC2) and ANOVAs on the bill and tarsus length measurements and on the bill shape index (Santiago, n = 33, Fogo, n = 27 and S. Nicolau, n = 18). The table shows the coefficient differences, lower and upper 95% confidence intervals, and adjusted p-value. Significant differences highlighted in bold.

		Coef. difference	**lower**	**upper**	**adjusted p-value**
PC1	Santiago-Fogo	-0.364	-1.287	0.560	0.616
	Santiago-S. Nicolau	**-1.089**	**-2.132**	**-0.047**	**0.039**
	S. Nicolau-Fogo	0.726	-0.357	1.809	0.251
PC2	Santiago-Fogo	0.171	-0.375	0.718	0.735
	Santiago-S. Nicolau	**-1.626**	**-2.243**	**-1.008**	**<0.001**
	S. Nicolau-Fogo	**1.797**	**1.156**	**2.438**	**<0.001**
Bill length	Santiago-Fogo	-0.059	-0.373	0.256	0.896
	Santiago-S. Nicolau	**0.689**	**0.335**	**1.042**	**<0.001**
	S. Nicolau-Fogo	**0.747**	**0.377**	**1.118**	**<0.001**
Tarsus length	Santiago-Fogo	0.010	-0.517	0.537	0.999
	Santiago-S. Nicolau	**-1.100**	**-1.692**	**-0.507**	**<0.001**
	S. Nicolau-Fogo	**-1.110**	**-1.727**	**-0.492**	**<0.001**
Bill shape	Santiago-Fogo	0.018	-0.002	0.039	0.094
	Santiago-S. Nicolau	**-0.033**	**-0.056**	**-0.010**	**0.003**
	S. Nicolau-Fogo	**0.051**	**0.027**	**0.076**	**<0.001**

Moult stages

I recorded moult stages during the breeding season, from September to January (Table S1). For adult birds, I recorded moult stages for two individuals in September, 17 in October, 32 in November, 49 in December and 13 in January (Table S1, Figure 5.4). Because moult stage was only recorded for two adult individuals in September, I only report results for the period between October and January (Figure 5.4).

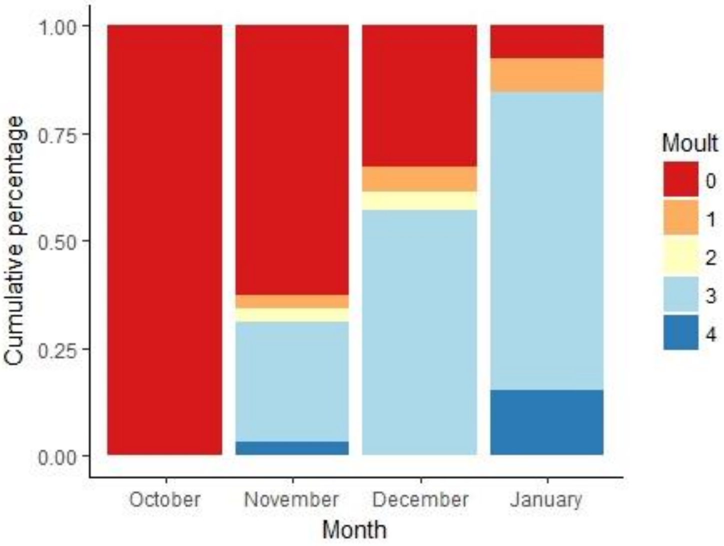


Figure 5.4 Moult stages in the Cape Verde warbler, throughout the course of the breeding season, from 0 (not moulting) to 4 (moult ending). Each bar shows the relative percentages of birds undergoing each moult stage for a given month: October (n = 17), November (n = 32), December (n = 49) and January (n = 13). Data were collected in 2013 and 2014. Moult stages are as follows: 0 = not moulting, 1 = moult starting, 2 = body moult, 3 = flight feather moult, 4 = moult ending.

5.5 Discussion

I found no significant overall body size divergence between the Cape Verde warbler populations of Santiago and Fogo (Table 5.4, Figure 5.3). However, there is significant divergence in tarsus length, bill length and bill shape between S. Nicolau and the other two populations (Table 5.4, Figure 5.3). This pattern matches that of the neutral genetic variation observed for this bird, which revealed a divergence between the three populations but especially large divergence between S. Nicolau and the other two populations, and is consistent with the colonisation and evolutionary history of the three populations (Batalha *et al.* 2017). The population of S. Nicolau became isolated from the two Sotavento populations circa 199,000 years ago, while Santiago and Fogo became isolated about 165,000 years ago, and there is little to no gene flow between them (Batalha *et al.* 2017). This pattern is also consistent with the hypothesis that the Cape Verde warbler previously occupied the more eastern islands in a stepping stone type colonisation, where divergence would have been initiated (Cibois et al. 2011; Spurgin et al. 2014).

Genetic drift is a strong evolutionary force acting on populations that have undergone bottlenecks (Hartl & Clark 1997), and it is the null hypothesis against which selection can be tested, i.e. it can only be discarded when selection is observed (Orr 1998). Several studies aimed at discerning the relative roles of neutral and adaptive forces in driving morphological divergence found no support for the action of selective forces when such divergence matched neutral genetic diversity patterns (Miller & Lambert 2004; Spurgin *et al.* 2014). These results are consistent with expectations derived from drift, because the pattern of morphological divergence seen in the Cape Verde warbler matches that of neutral genetic divergence. This means that, even if selection is occurring, it is being overridden by neutral forces in these traits (Gonzalez-Quevedo *et al.* 2015; Grueber *et al.* 2013; Miller & Lambert 2004; Spurgin *et al.* 2014). The results are more consistent with the occurrence of a bottleneck on the population of S. Nicolau, as previously inferred from genetic data (Batalha *et al.* 2017), which could have led to drift in morphological traits. For example, reductions in tarsus length have been seen following introduction of five New Zealand blackbird populations, followed by bottleneck effects (Blackburn *et al.* 2013). However, an interaction of drift and selection in shaping morphological divergence across islands cannot be completely ruled out (James 1982). Interactions between neutral and adaptive evolutionary forces are difficult to disentangle and usually need long-term studies (Aleixandre *et al.* 2013; Grueber *et al.* 2013; van Oosterhout *et al.* 2006). Alternatively, morphological differences between individuals of S. Nicolau and those of the other two populations can simply reflect phenotypic plasticity (Larsson & Forslund 1991; Przybylo *et al.* 2000).

Our results are in accordance with biogeographical patterns previously reported for other Cape Verde species. Divergence between populations of the Barlavento and Sotavento groups of islands has been reported for another bird, the Common kestrel *Falco tinnunculus* (Bourne 1955; Hille *et al.* 2003). This bird is divided in two subspecies in Cape Verde, *Falco tinnunculus neglectus* inhabiting the northern Barlavento group of islands, and *Falco tinnunculus alexandri* found on the southern Sotavento island group (Bourne 1955; Hille *et al.* 2003). One possible explanation for this biogeographical pattern is that distance between island groups and wind patterns can prevent dispersal more strongly between the Barlavento and Sotavento groups of islands than between islands of the same group (Correia 1996; Hille *et al.* 2003). Morphological descriptions for other taxa in Cape Verde are scarce, but the same pattern of morphological divergence between northern and southern island groups can be seen in several reptile species (Arnold *et al.* 2008; Miralles *et al.* 2011; Vasconcelos *et al.* 2012).

Size differences in bird tarsi and bills may reflect a different use of habitat structures or food resources (Grant 1965; Miles & Ricklefs 1984; Ricklefs & Cox 1977). Tarsus length is a heritable trait and therefore can be subjected to selection (Alatalo & Lundberg 1986). Bird species which feed in a hanging position, use unstable perches or climb tree trunks seem to have shorter tarsi (Grant 1965). Hence, from a habitat use perspective, differences in tarsus size between the birds of S. Nicolau, where males have longer tarsi, and the other two islands might be related to the fact that these birds seem to use different plant species on S. Nicolau, i.e. mostly reed patches (Chapter 4). Variation in the shape and size of bird bills is correlated with the food resources used (Bowman 1961; Grant 1985; Grant 1965; Schluter & Grant 1984a). In the Galapagos finches, variation in bill size and shape among populations on different Galapagos islands is related to the size of the food resources used by the finches (Grant 1985; Grant *et al.* 1985; Grant 1986; Schluter & Grant 1984b). A correlation between bill length and average size of prey eaten can be seen in several other insular birds species (Baldwin 1953; Grant 1965). The differences in bill shape between the warblers of S. Nicolau and those of Santiago and Fogo might be an adaptation to different food resources, or might simply be the result of drift. However, at this point I can only speculate about possible causes for these differences, and research directed specifically to the diet and foraging behaviour of these birds is needed to verify these hypotheses.

Selective forces acting in the wild are often strong and variable in space and time (Bell 2008; Endler 1986). In animal populations, evolutionary responses to selective forces can fluctuate over short time scales (Bell 2010; Hill *et al.* 1991). Fluctuating selection and pressures created by environmental instability have been proposed to explain rapid evolution of morphological traits (Benkman & Miller 1996; Millet *et al.* 2015; Thompson 1998). Rapid ecological evolution, i.e. that which occurs in timespans of less than a century, has been described for other birds species that show adaptive radiations in archipelagos (Clegg *et al.* 2008; Grant 1985; Thompson 1998). Rapid changes in bill shape and size in *Geospiza* finches and *Zosterops* white-eyes have been linked to climatic events and proposed to occur because of the changes in food resources induced by those climatic events (Clegg *et al.* 2008; Grant 1985; Thompson 1998). While fluctuating selection has been confirmed as a mechanism driving morphological changes, this has been documented with long term studies and/or links to environmental changes (Bell 2010; Clegg *et al.* 2008; Grant 2008). The time scale of this study, one calendar year, is not enough to make inferences or speculations about a possible role of fluctuating selection and rapid evolution in driving divergence between the populations of Cape Verde warbler. Further studies could confirm or discard this hypothesis.

Conclusion

Our results indicate that there is sexual dimorphism with respect to body size in the Cape Verde warbler (Tables 5.2 and 5.3, Figure 5.2), even though, as in many passerines there is a large overlap between the sexes. This pattern is seen in most *Acrocephalus* warbler species (Cramp & Perrins 1992; Svensson 1992). I also report the first records of morphological measurements of live Cape Verde warblers for around 100 years (Cramp & Perrins 1992; Dohrn 1871; Garcia-del-Rey 2016; Keulemans 1866) and the first records of moult patterns (Garcia-del-Rey 2016). Data were collected during the breeding seasons of 2013 and 2014, and juveniles as well as adults were measured. I also observed some nests with chicks or eggs during the same period. This confirms that the main breeding season of the Cape Verde warbler falls during the months following the start of the rain season in Cape Verde (usually late August – early September), on all islands (Bourne 1955; Correia 1996; Garcia-del-Rey 2016; Hazevoet 1995). The results indicate that adult Cape Verde warblers undergo a post-breeding moult which lasts at least from October to January (Figure 5.4). This is consistent with the pattern observed in most resident songbirds (Leisler & Schulze-Hagen 2011).

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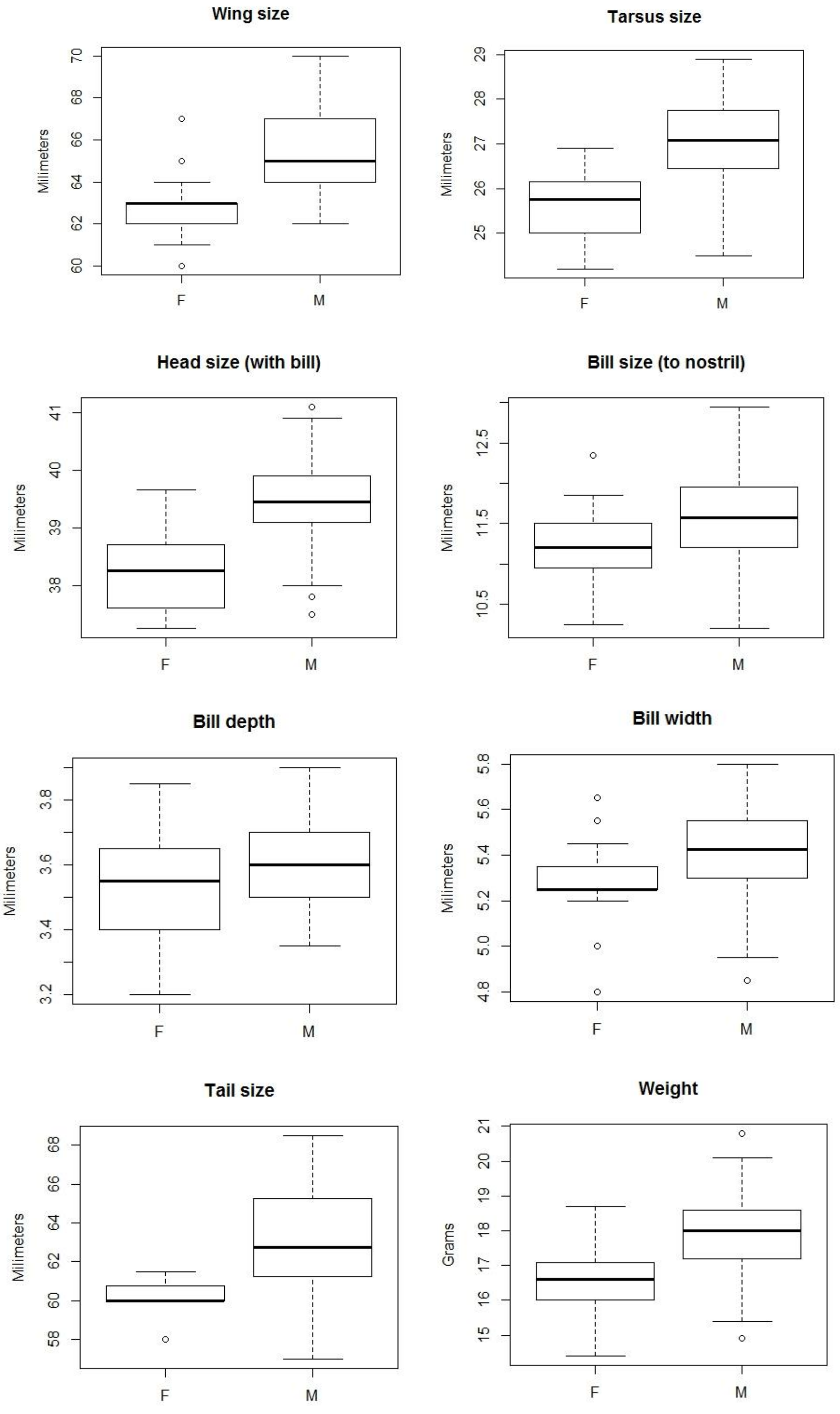
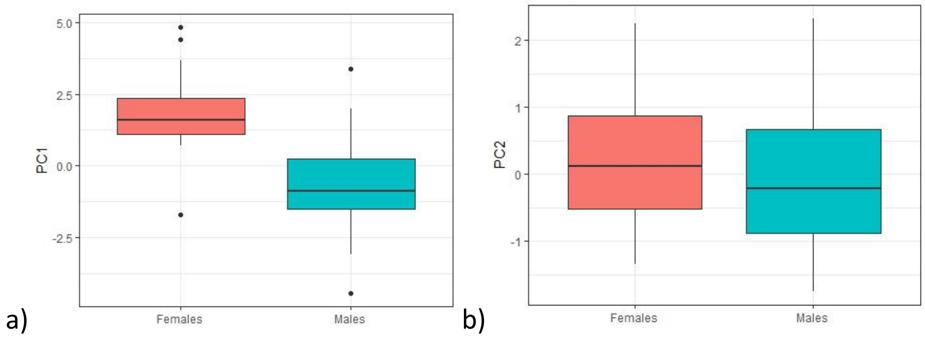
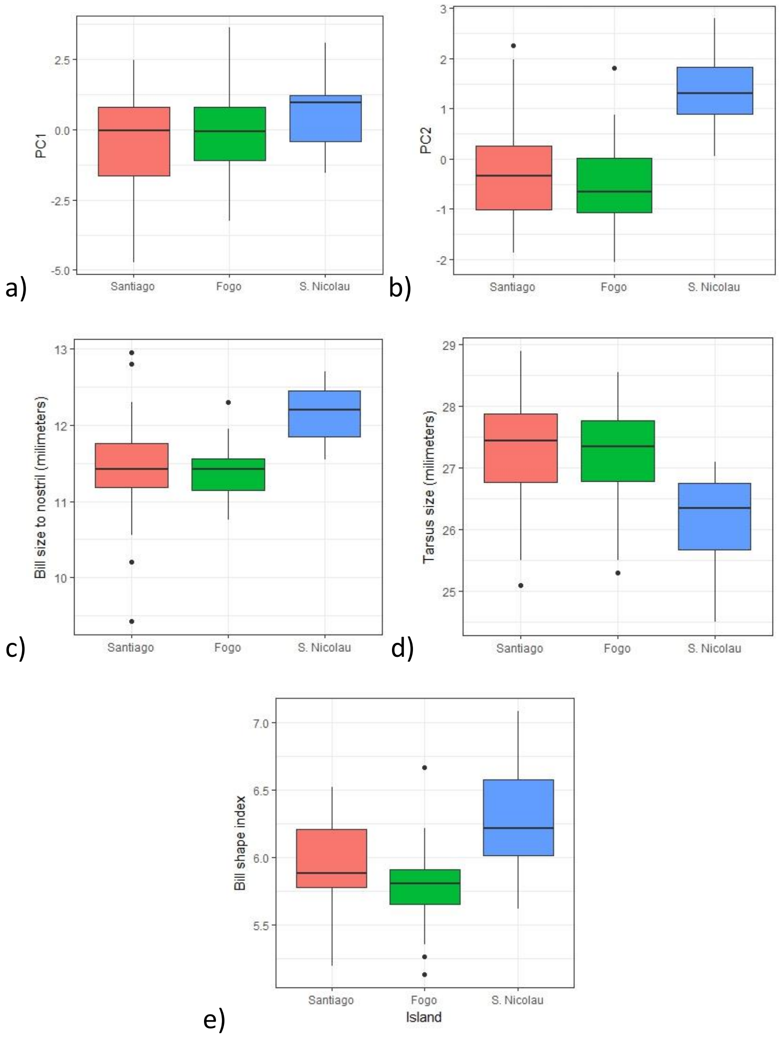


Supplementary figure S5.1 Boxplots of morphological measures taken on the Cape Verde warbler during the breeding seasons of 2013 and 2014, including only adult birds (F = females and M = males) from Santiago, Fogo and S. Nicolau.



Supplementary figure S5.2 Sexual dimorphism in the Cape Verde warbler *Acrocephalus brevipennis*: boxplots of principal component scores of PC1 (a) and PC2 (b); groups correspond to adult males, n = 78 and adult females, n = 29.



Supplementary figure S5.3 Island differences in adult males Cape Verde warblers *Acrocephalus brevipennis*: principal component scores of PC1 (a), PC2 (b); measurements of bill size to nostril (c) and tarsus size (d); bill shape index scores (e). Boxplots of Santiago, n = 33, Fogo, n = 27 and S. Nicolau, n = 18.

Chapter 6

General discussion

Sunset over Fogo island, seen from Serra Malagueta, Santiago. Photo taken in November 2014

6.1 General discussion

In this thesis I assess genetic and phenotypic divergence between the three remaining populations of the endangered Cape Verde warbler, and consider the conservation implications of the results. Divergent, isolated populations can require different management strategies (Linnell *et al.* 2008; Sylven 2003) because intrinsic and extrinsic factors can influence viability in a different way for each population. In fragmented populations factors such as population numbers, genetic diversity, habitat use, local behavioural or morphological adaptations, levels of predation or parasite prevalence all interact to determine each population's viability (Dalton *et al.* 2013; Howland *et al.* 2016; Michaux *et al.* 2004; Stangel *et al.* 1992). Therefore, the first step in an effective species conservation plan is to understand potential different requirements of different populations. In this final chapter, I discuss my findings collectively and outline ideas for future research.

6.1.1 Population divergence

Genetic factors can contribute significantly to extinction risk in the wild (Frankham 2005; Frankham & Ralls 1998; Saccheri *et al.* 1998; Spielman *et al.* 2004), particularly when populations are isolated or arranged in a meta-population structure (Saccheri *et al.* 1998). Neutral markers can be used rapidly and efficiently to assess population genetic diversity, divergence and past and ongoing bottlenecks (Frankham 1998; Padilla *et al.* 2015; Ramstad *et al.* 2013; Spurgin *et al.* 2014; Wright *et al.* 2014). In Chapter 2, I used mitochondrial DNA and microsatellite markers to show that the three populations of the Cape Verde warbler exhibit a gradient of diversity, with the population of the largest island, Santiago, having the most diversity and the smallest population of S. Nicolau having the least diversity. Divergence between populations based on microsatellites and mitochondrial DNA was medium to large (microsatellite F_{ST} 0.06 Santiago – Fogo to F_{ST} 0.28 Fogo – S. Nicolau; cytochrome b F_{ST} 0.26 Santiago – Fogo to F_{ST} 0.45 Fogo – S. Nicolau). This and the recent divergence times (less than half a million years ago) collectively suggest relatively recent isolation between the three populations, with little to no gene flow, which is consistent with the initial phases of allopatric speciation (Coyne & Orr 2004; Orr & Smith 1998; Price 2008).

Culturally inherited behavioural traits can evolve rapidly due to stochastic or selective forces, and this rapid evolution can reinforce neutral genetic divergence among populations (Grant & Grant 1996; Kenyon *et al.* 2016; MacDougall-Shackleton & MacDougall-Shackleton 2001; Price 2008). In other words, if drift is driving divergence between vertebrate populations, culturally inherited traits can augment and advance the divergence process. In Chapter 3, I determined song divergence between the Cape Verde warbler populations, and assessed whether this divergence could play a role in reproductive isolation, thereby reinforcing population divergence. I found no significant differences in the spectral traits of song between the three populations, i.e. there was no difference in the frequencies, entropy and duration of song between the three populations. However, there was some indication that the structural elements of the songs were arranged differently between the three populations, especially when comparing S. Nicolau with the other two populations. There was also no difference in the behavioural or song reaction of the birds of each population to songs of different origins. Overall, I found no evidence that the individuals from any population of the Cape Verde warbler discriminate between birds from different populations based on song. This result contrasts with those observed in a number of other species, where individuals discriminate against songs of males of different origins (Grant & Grant 2002; Irwin *et al.* 2001; Mortega *et al.* 2014).

In Chapter 4, I explored habitat requirements of the Cape Verde warbler in the three islands where it exists and assessed if there were differences between them. For animals, structural vegetation traits are an important feature in determining habitat preferences (Odum 1971; Wildi 2013). Dense green vegetation cover that persisted year-round predicted the wider areas occupied by the warbler on all three islands. However, no topographic or structural vegetation traits predicted fine scale presence of the warbler on any of the islands. Interestingly, plant species composition differed between sites occupied and unoccupied by the warbler, and also differed between the three islands. Generally speaking, on Santiago and Fogo, the warbler uses plantations and forests, whereas on S. Nicolau it is mostly restricted to dense reed *Arundo donax* beds and mango *Mangifera indica* trees, but is absent from plantations and forests. The reasons for this difference are unknown at the moment.

Morphological differences have been historically used to define subspecies, and comparing morphological and neutral genetic divergence can help disentangle the relative roles of neutral and evolutionary forces in driving population divergence (Clegg *et al.* 2002; Clegg & Phillimore 2010; Potvin & Clegg 2015). Thus, any assessment of population divergence would not be complete without examining morphological differences. In Chapter 5, I examined differences in morphological traits between the three populations of the Cape Verde warbler. The results indicate that birds of S. Nicolau have longer, narrower bills and shorter tarsi than the birds of Santiago and Fogo, which do not differ between each other.

S. Nicolau

While I did not find evidence of song divergence between the three populations, it is clear that neutral genetic markers, habitat preferences and morphological traits differ between them. In all measured traits, the small isolated population of S. Nicolau sets apart from Santiago and Fogo, which confirms its uniqueness. It is the least genetically diverse population, and it does not share mitochondrial DNA alleles with Santiago or Fogo. The results of Chapter 2 also indicate that S. Nicolau became isolated from the other two populations earlier (199,000 year ago) than they were isolated from each other (165,000). Finally, there is strong evidence of a recent or current bottleneck in this population, and the population size is very small. Estimates of effective population size using standard methods (Spurgin *et al.* 2014) were not possible (for any of the populations), and information about the past size of the populations is not available, which makes determining the exact strength of this bottleneck difficult. However, there is anecdotal evidence that the whole archipelago has suffered from droughts and changes in land management during the last century (Hazevoet 1995), which might have caused habitat loss and therefore a decrease in population size and consequently loss of genetic diversity. There is no statistical evidence for song divergence in this population, except for the fact that songs sung by S. Nicolau birds are slightly longer and seem to include different elements, e.g. more trills, that those of birds of the other two populations. The habitat used by S. Nicolau warblers, while structurally similar, is composed of different plant species than warbler habitat on the other islands. Furthermore, unlike the warblers of Santiago and Fogo, those of S. Nicolau are not using all potentially suitable habitat on this island. Finally, they have shorter tarsi and longer, narrower bills than the birds of the other two islands. Collectively, my results indicate that the warblers of S. Nicolau differ more from those of Santiago and Fogo than those of these two islands between themselves.

6.1.2 Conservation implications

Conservationists often focus on preserving "irreplaceable" populations, i.e. those with unique characteristics (Brooks *et al.* 2006). The observed significant diverge of allele frequencies at mitochondrial DNA and microsatellites between the three populations of the warbler are sufficient to consider each one of them as a different management unit, sensu Moritz (1994), because they diverge significantly at mitochondrial DNA. Currently, they should not be considered different evolutionarily significant units because they are not reciprocally monophyletic for mitochondrial DNA alleles (Moritz 1994). This recommendation is reinforced by the fact that the warblers differ in habitat use and morphology on the three islands. Notably, habitat is a key element to be taken into account in conservation plans. This is not only because changes to habitat can have a direct and immediate effect on a population (Butchart *et al.* 2010; Pereira *et al.* 2010), but also because the geographical location of critical habitat determines who owns them, and consequently influences how they can be managed. Therefore, I suggest that habitat-directed conservation measures should be specifically tailored for each island, taking into account the different plant species composition and geographic distribution of suitable areas for the warbler.

Collectively, the results of this thesis show that the small isolated population of S. Nicolau is different from the populations of Santiago and Fogo in terms of neutral genetic diversity, habitat use and morphological traits. This population is very small, i.e. 12-25 breeding pairs (Batalha *et al.* 2017), and most territories were found outside the protected area of the Natural Park of Monte Gordo. Taken together, this implies that the conservation of this population should be prioritised, but that this can be difficult to achieve. Genetic rescue by translocation of individuals from Santiago or Fogo could be an option (Weeks *et al.* 2011), but practitioners could risk losing the unique genetic diversity found in this population, or creating outbreeding problems (Frankham 2015; Frankham *et al.* 2011). Therefore, all potential benefits and problems derived from using this approach should be carefully considered. Finally, at the moment it is unknown if the population bottleneck on S. Nicolau is simply due to founder effects or if it is the result of environmental pressures such as the droughts that devastated the archipelago during the last century (Hazevoet 1995; Sena-Martins & Moreno 1986). It would be important to explore possible causes of the bottleneck, for example using climatic data, and even model future scenarios to understand if this population can be facing potential additional bottlenecks.

6.1.3 General conclusions and further work

The research presented in this thesis provides insight into population divergence in the Cape Verde warbler and bears important implications for its conservation. No functional markers have been assessed in this thesis, but a study of toll-like receptor immune gene diversity is underway with collaborators. The study of immune gene diversity, perhaps linked to differences in pathogen prevalence on the three populations, could provide additional insight into selective forces or conservation threats impacting the warbler (Gonzalez-Quevedo *et al.* 2015; Radwan *et al.* 2010; Spurgin & Richardson 2010). Regarding estimates of divergence time, the results have high 95% HPD intervals because there is very little divergence in mitochondrial sequences used. In other words, while the geometric averages of divergence time are 165-199 kya, it is possible that the three populations diverged from each other sometime between 52-615 kya. A possible solution for this would be to sequence more mitochondrial genes, but for the purpose of this study, i.e. defining conservation management units, the use of the cytochrome b was sufficient.

Bird song, as cue for mate recognition, can indicate reproductive isolation (Mason *et al.* 2017; Slabbekoorn & Smith 2002). The CVW songs do not differ between islands and birds do not respond differently to songs of different populations of origin, suggesting that song does not play a role in reproductive isolation in this species. However, small sample size coupled with high inter-individual variability in the CVW could have obscured the results of playback tests. Furthermore, in structurally complex and individually varied songs like in the CVW, differences in song structure can only be reliably assessed by examining a sufficiently large number of songs per individual and population (Mortega *et al.* 2014). Because the Cape Verde warbler sings very irregularly and infrequently, it was not possible to obtain such a sample size within the limitations of the fieldwork period. Further expeditions could be undertaken to record more songs and continue this part of the study.

Apart from reed on S. Nicolau, the warbler uses mostly vegetation used for agriculture and forests, i.e. exotic species generally located on privately owned land. Therefore, any management strategy should take this into account and aim to promote a harmonious coexistence between the warbler and humans. Removal of exotic plants, e.g. Spanish flag *Lantana camara*, has been advocated in the past to preserve and restore native flora in Cape Verde. However, this could have negative impacts in the warbler, particularly on S. Nicolau. It would be advisable to carefully assess the benefits for native flora and potential disadvantages for the warbler before any removal action takes place. Alternatively, when such assessments are costly in terms on time and money, it seems plausible to find a compromise solution like the one implemented on Serra Malagueta Natural Park in 2014: biologists removed Spanish flag shrubs only from the areas where no warbler nests had been found (J. Mascarenhas, pers. comm.).

Habitat analysis results suggest that the warbler is not using the entire available suitable habitat on S. Nicolau, i.e. that warbler habitat has not reached carrying capacity (Odum 1971). This could simply be a recent consequence of the bottleneck this population has been through (Batalha *et al.* 2017). Alternatively, it could be linked to potential inbreeding depression or external biotic forces such as pathogens, predators or competitors (Frankham *et al.* 2009; Odum 1971). Predators, especially mammalians, have been linked to declines of endemic island birds, e.g. in New Zealand, Hawaii or some Atlantic islands (Hilton & Cuthbert 2010; McLennan *et al.* 1996; Wilson *et al.* 1998). However, they have a stronger impact on birds with slow rates of growth, long generation time or that nest in holes (O'Donnell 1996; Owens & Bennett 2000). Thus, it is advisable that inbreeding depression and external threats are assessed to better understand their potential impact on this population, as well as to inform efficient conservation measures.

Morphological differences between populations have been linked to the action of selection environments (Grant 1965, 1986; Schluter & Grant 1984), these studies relied on the collection of long term data or because environmental causes of selection were observed as they occurred. To determine if, in addition to drift and bottleneck effects, selection has a role in shaping differences in tarsus and bill size in the S. Nicolau warblers, regular data collection should be undertaken. This could be part of an integrated monitoring plan, involving for example bird ringing and measuring, blood sampling, nest and territory checks, habitat surveys, assessment of predators and pathogens and observations of feeding behaviour.

Finally, a consistent issue during this work is the sparsity of data on territory size, diet, predation, life cycle, breeding biology and reproductive success on the Cape Verde warbler (BirdLife International 2016; Garcia-del-Rey 2016). Successful conservation plans need to take such species requirements into account (Groom *et al.* 2006; Lindenmayer & Burgman 2005). In this thesis, I have taken a first step by examining habitat preferences of the warbler on the three islands, but this work needs to be further developed and incorporate other potentially important factors, such as predators, pathogens and competitors. I emphasize the need for this research to be undertaken outside the Natural Park areas, especially on S. Nicolau, as most of the few existing territories are located outside this area.

Final conclusion

In conclusion, this thesis provided a practical example of how examining data from multiple traits is important to understand population divergence and implications in its conservation, because each trait can evolve in different ways and be subjected to different evolutionary forces and environmental pressures. The findings presented here indicate that the three populations of Cape Verde warbler differ in genetic, habitat and morphological traits and should be considered different conservation management units. Nevertheless, further research directed to the specific needs of each population is needed to ensure the drafting of a successful conservation plan for this species, and especially for the small and unique population of S. Nicolau.

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Appendix I

Conservation, ecology and genetics of the Cape Verde warbler

Acrocephalus brevipennis

Report on fieldwork in Cape Verde

November 2013 – January 2014

Colour-ringed juvenile Cape Verde warbler Acrocephalus brevipennis, *Fogo. Photo taken in December 2013.*

CONSERVAÇÃO, ECOLOGIA E GENÉTICA DA FELOSA DE CABO VERDE *Acrocephalus brevipennis*

Relatório sobre o trabalho de campo em Cabo Verde
Novembro 2013 – Janeiro 2014

CONSERVATION, ECOLOGY AND GENETICS OF THE CAPE VERDE WARBLER *Acrocephalus brevipennis*

Report on fieldwork in Cape Verde
November 2013 – January 2014

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Abril de 2014 | April 2014

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Financiamento | Funding: Fundação para a Ciência e Tecnologia, University of East Anglia, The Rufford Foundation, BirdLife International, African Bird Club & Royal Society for the Protection of Birds.

Apoio em Cabo Verde | Cape Verde partners: Direcção Geral do Ambiente (DGA), Instituto Nacional para a Investigação e Desenvolvimento Agrário (INIDA), Parque Natural da Serra Malagueta, Parque Natural do Fogo, Parque Natural de Monte Gordo, Biosfera I & Sociedade Caboverdiana de Zoologia.

Apoio internacional | International support: Sociedade Portuguesa para o Estudo das Aves

Foto de capa | Cover photo: felosa de Cabo Verde *Acrocephalus brevipennis* juvenil anilhado em Fogo (foto de Josh Jenkins Shaw) | juvenile Cape Verde warbler *Acrocephalus brevipennis* ringed on Fogo (photo by Josh Jenkins Shaw)

Agradecimentos especiais | Special thanks: David J. Wright, pelos comentários construtivos que melhoraram este relatório | David J. Wright, for the constructive comments which improved this report.

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Citação | Citation: Batalha, H. (2014). Conservation, ecology and genetics of the Cape Verde warbler *Acrocephalus brevipennis*: Report on fieldwork in Cape Verde, November 2013 – January 2014. Unpublished report, University of East Anglia, Norwich, UK.

Autorização | Permit: n ° 36/2013, Direcção Geral do Ambiente, Achada Sto. António, CP 332-A, Praia, Santiago, Cabo Verde

Abril de 2014 | April 2014

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1. Introdução | Introduction

A felosa de Cabo Verde (*Acrocephalus brevipennis*) é uma ave endémica deste arquipélago, classificada com o estatuto de conservação "em perigo" pela IUCN (BirdLife International 2013). Até recentemente, pensava-se que esta espécie se encontrava circunscrita à ilha de Santiago. Em 1998 e 2004 novas populações foram descobertas em S. Nicolau e Fogo, respectivamente (Hazevoet *et al.* 1999; Hering & Hering 2005). A população global desta espécie está estimada pela IUCN em apenas 1000-1500 aves (BirdLife International 2013). As relações filogenéticas entre elas ainda não foram estudadas. Diferenças entre as populações, nomeadamente genéticas, ecológicas ou comportamentais, podem prejudicar o sucesso de medidas de conservação (Phillimore *et al.* 2008). Para a preservação a longo prazo desta espécie, as diferenças entre as populações devem ser tidas em conta nos planos de conservação. Para avaliar estas diferenças, recolhemos amostras de sangue e medidas morfométricas e fisiológicas de 95 indivíduos pertencentes às três diferentes populações, entre Novembro de 2013 e Janeiro de 2014. Simultaneamente, identificámos cada ave individualmente com anilhas de metal e cor e recolhemos dados de canto das aves e habitat onde as aves foram encontradas. Estes dados serão agora analisados com recurso a técnicas moleculares e estatísticas para avaliar se existem diferenças entre as populações. Neste relatório apresentamos os dados recolhidos durante esta época de campo, avaliamos até que ponto os objectivos definidos foram alcançados e propomos direcções futuras para dar seguimento ao trabalho iniciado.

The Cape Verde warbler *Acrocephalus brevipennis* is an endemic bird of this archipelago classified as "endangered" (BirdLife International 2013). Until recently, it was thought that this species was confined to the island of Santiago. In 1998 and 2004 new populations were discovered on S. Nicolau and Fogo, respectively (Hazevoet *et al.* 1999; Hering & Hering 2005). Population sizes are estimated to be between just 1000 and 1500 mature individuals (BirdLife International 2013). Additionally, phylogenetic relationships between populations have not yet been assessed. Genetic, ecological or behavioral differences between populations can impair the success of conservation measures (Phillimore *et al.* 2008). For the long-term conservation of this species, this should be taken into account in conservation plans. To evaluate these differences, we collected blood samples and morphometric and physiological measurements of 95 individuals belonging to the three different populations, between November 2013 and January 2014. Simultaneously, we identified each bird individually with metal and colour-rings and collected data on bird song and the habitat where they were found. These data will be now analysed with molecular and statistical techniques to assess the extent of the differences between populations. In this report, we present the data collected during this field season. We evaluate if the planned goals have been reached and then propose new activities to follow up the work initiated.

2. Objectivos | Aims

Tendo em conta o objectivo principal dste projecto, para a primeira época de campo (Novembro 2013 a Janeiro 2014) foram definidos os seguintes objectivos específicos:

To achieve the main aims of this project, we defined as specific goals of the first field season (November 2013 to January 2014) the following:

1) Construir colaborações com entidades nacionais no sentido de iniciar e desenvolver o projecto
    - Build collaborations with local stakeholders to initiate and develop the project

2) Iniciar o treino de técnicos locais para dar continuidade à monitorização da espécie
    - Start training local technicians to ensure that the species monitoring will continue

3) Obter amostras de sangue de um mínimo de 30 aves por ilha
    - Obtain blood samples of a minimum of 30 birds per island

4) Recolher dados de morfologia, de muda, época de reprodução e parasitas
    - Record data on biometrics, moult, breeding stage and parasites

5) Gravar o canto de um mínimo de 10 machos por ilha
    - Record songs of a minimum of 10 male birds per island

6) Fazer um primeiro reconhecimento das características do habitat da espécie
    - Make a first assessment of habitat features

7) Testar um método adequado para fazer censos dos números da população
    - Test an adequate method to census population numbers

3. Actividades e resultados do projecto | Project activities and outcomes

3.1. Colaboração com entidades nacionais | Collaboration with national stakeholders

O trabalho de campo para o estudo e conservação da felosa de Cabo Verde *Acrocephalus brevipennis* teve lugar no final da época reprodutora, com início a 17 de Novembro de 2013 e final dia 28 de Janeiro de 2014. Desde a chegada a Cabo Verde, a equipa de campo contactou sempre todas as entidades nacionais no sentido de pedir a sua autorização para realizar os trabalhos e manter estas entidades informadas do desenvolvimento dos mesmos. Vários técnicos e directores do INIDA (Instituto Nacional de Investigação e Desenvolvimento Agrário), Parque Natural da Serra Malagueta, Parque Natural do Fogo e Parque Natural de Monte Gordo acompanharam a equipa em algumas saídas de campo, em função da conjugação da sua disponibilidade com a localização e datas dos trabalhos. Colaborámos também com a ONG Biosfera I no sentido de incorporar a nossa metodologia de trabalho no esquema nacional de anilhagem de aves que a mesma está a desenvolver em conjunto com a DGA (Direcção Geral do Ambiente). Além disso, graças ao financiamento do African Bird Club, foi possível recrutar uma excelente bióloga Cabo Verdeana (Jaelsa Moreira) que trabalhou como assistente de campo neste projecto durante 2 meses. Todas as pessoas envolvidas mostraram um grande interesse em aprender, acompanhar e ajudar a realização do trabalho, o que se reflectiu num excelente resultado final.

> The fieldwork for the study and conservation of the Cape Verde Warbler Acrocephalus brevipennis took place at the end of the breeding season, starting on November 17th, 2013 and ending January 28th, 2014. After arriving in Cape Verde, we contacted all national authorities for permission to undertake the work and kept them informed of progress throughout the fieldwork. Where possible we took local stakeholders to the field with us to follow the work. Several technicians and directors from the National Institute for Agrarian Research and Development (INIDA) and Natural Parks of Serra Malagueta, Fogo and Monte Gordo accompanied the team on field trips, whenever possible. We also collaborated with the NGO Biosfera I to incorporate our methodology in the national bird ringing scheme that is being developed jointly with the General Direction for the Environment (DGA). Moreover, thanks to funding from the African Bird Club, we hired a Cape Verdean biologist (Jaelsa Moreira) who did an excellent work as a field assistant on this project for two months. All involved stakeholders showed great interest in learning and helping to carry out the work, which contributed to the succes of this fieldwork.

3.2. Treino de técnicos locais | Training of local technicians

É fundamental, para o sucesso de qualquer projecto de conservação *in situ* a longo prazo, que os parceiros locais estejam envolvidos no mesmo desde o início. Assim, um dos objectivos deste projecto de doutoramento é formar uma rede de colaboradores em Cabo Verde que possam dar continuidade ao projecto, e desenvolver as competências técnicas dos biológos que fazem o seguimento ecológico das felosas de Cabo Verde. Neste sentido, procurámos dar formação inicial em técnicas de monitorização de aves que envolvem anilhagem a vários técnicos do INIDA, Parque Natural da Serra Malagueta, Parque Natural do Fogo e Parque Natural de Monte Gordo. Os técnicos tiveram alguma formação inicial em montagem e desmontagem de redes verticais, extracção de aves das redes, manuseamento e anilhagem das aves, recolha de parâmetros biométricos e significado biológico dos mesmos (por exemplo, muda, pelada de incubação, etc). Além destes técnicos, também a assistente de campo cabo verdeana Jaelsa Moreira teve oportunidade de desenvolver bastante as suas competências técnicas em todas as fases do processo de anilhagem, bem como no seguimento ecológico da felosa de Cabo Verde no campo.

> A key aspect of the success of long-term *in situ* conservation projects is the involvement of local partners from the start. Hence, one of the main aims of this project is to build a network of collaborators in Cape Verde who can continue the project, and to increase the technical skills of the local biologists monitoring the birds. To do so, the team included local technicians in our field trips, so that they would appreciate the avian monitoring techniques used. We gave basic training in bird ringing to technicians of INIDA and Natural Parks of Serra Malagueta, Fogo and Monte Gordo. The technicians had initial training in setting up and closing mist-nets, extracting birds from nets, handling and ringing, collecting biometric parameters and about the biological significance of these measurements (ie, moult, brood patch, etc.). In particular, the Cape Verdean field assistant Jaelsa Moreira developed her technical skills in all aspects of bird ringing, as well as in monitoring the Cape Verde warbler.

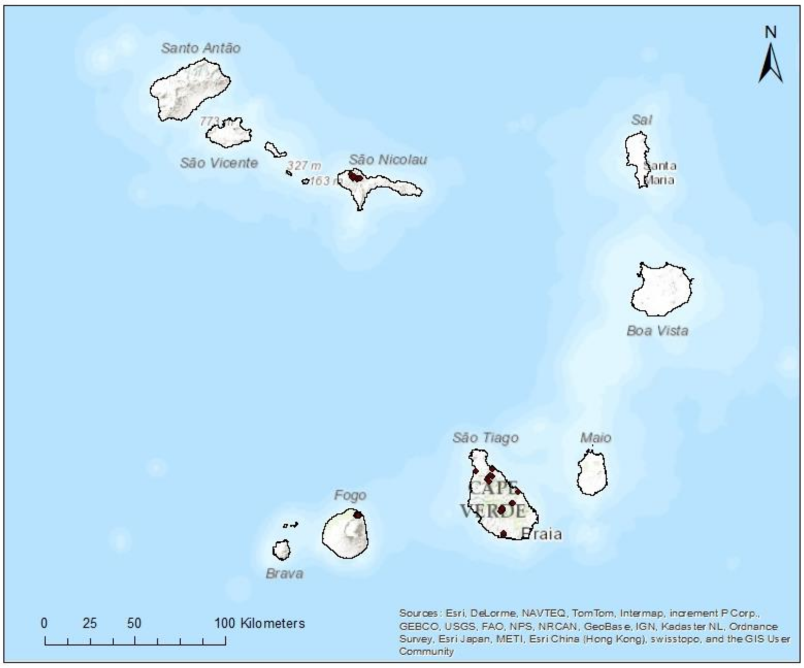
3.3. Amostras de sangue | Blood samples

Definiu-se como objectivo conseguir um mínimo de 30 amostras de sangue por população, porque é o número mínimo recomendado para estudos que pretendam ilustrar variabilidade genética e diferenças entre populações. Um número de amostras inferior poderá deturpar os resultados. A equipa capturou, anilhou e recolheu amostras de sangue e dados biométricos de um total de 95 felosas de Cabo Verde (Table I). Destas, 50 foram capturadas em Santiago, 30 em Fogo e 15 em S. Nicolau, em 62 locais diferentes (Figure 1). Para identificar as aves amostradas, cada uma recebeu uma anilha de metal numerada e uma combinação única de três anilhas de cor resistentes a radiação UV (Redfern & Clark 2001; ver Table I e Anexo I). As amostras de sangue foram recolhidas por punctura da veia braquial. Adicionalmente foram anilhadas e medidas outras aves que caiam acidentalmente na rede e cujo tarso suportava anilhas do mesmo tamanho. Infelizmente uma ave morreu durante a fase de amostragem de sangue devido a um acidente, na ilha do Fogo (ver tabela XI no Anexo II: captura #68). Estavam presentes os quatros membros da equipa de trabalho, bem como o Dr. Alexandre Nesvsky e o técnico Herculano Dinis, do Parque Natural do Fogo, e o habitante de Monte Barro Nené, e foram feitos todos os possíveis para salvar a ave. Todas as outras aves foram libertadas ilesas imediatamente após o processamento. Mortes durante a anilhagem são muito raras (Sheldon *et al.* 2008; Spotswood *et al.* 2012), mas infelizmente podem acontecer até mesmo aos anilhadores mais experientes.

> We aimed to collect 30 blood samples per population, the minimum recommended number of samples for genetic studies assessing variability and differences between populations. A smaller number of samples may distort results. Our team captured, ringed and collected blood samples and biometric data from 95 Cape Verde warblers (Table I), 50 on Santiago, 30 on Fogo and 15 on S. Nicolau, in a total of 62 different locations (Figure 1). To identify the sampled birds, each received a numbered metal ring and a unique combination of three colour rings resistant to UV radiation (Redfern & Clark 2001; see Table I and Appendix I). Blood samples were collected from the the brachial vein. In addition, we measured and ringed other birds that were accidentally caught and whose tarsus could fit rings of the same size. Unfortunately one bird died during the sampling process due to an accident, but all other birds were released unharmed immediately after ringing. Such deaths are very rare during bird ringing (Sheldon et al. 2008; Spotswood et al. 2012) but, regrettably, can happen even to the most experienced ringers.

Table I – Número de felosas de Cabo Verde *Acrocephalus brevipennis* amostradas por ilha, após a época de reprodução de 2013, ordenadas por idade e sexo; F = fêmea, M = macho, N = total / Number of Cape Verde warblers *Acrocephalus brevipennis* sampled per island, after the breeding season of 2013, by age and sex; F = female; M = male, N = total.

	Idade / Age			
Sexo / Sex	Desconhecida Unknown	Juvenil Juvenile	Adulto Adult	**N**
Santiago 18/11/2013 - 14/12/2013	**2**	**9**	**39**	**50**
Centro / Centre	**1**	**5**	**22**	**28**
F	0	3	9	12
M	1	2	13	16
Norte / North	**0**	**0**	**15**	**15**
F	0	0	4	4
M	0	0	11	11
Sul / South	**1**	**4**	**2**	**7**
F	0	3	1	4
M	1	1	1	3
Fogo 19/12/2013 - 31/12/2013	**6**	**3**	**21**	**30**
F	1	3	4	8
M	5	0	17	22
S. Nicolau 04/01/2014 - 19/01/2014	**0**	**2**	**13**	**15**
F	0	1	2	3
M	0	1	11	12
Total por idade / by age	**8**	**14**	**73**	**95**

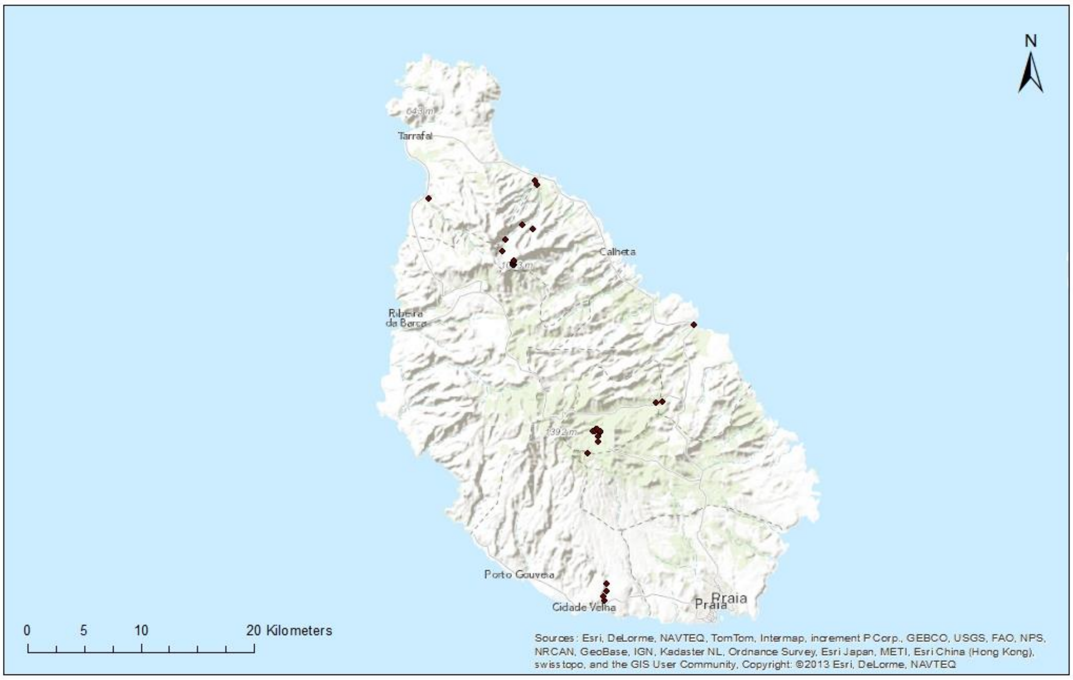


Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Figure 1 – Vista geral da distribuição dos locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis*, após a época de reprodução de 2013 / Overview of the sites where Cape Verde warblers *Acrocephalus brevipennis* were sampled, after the breeding season of 2013.

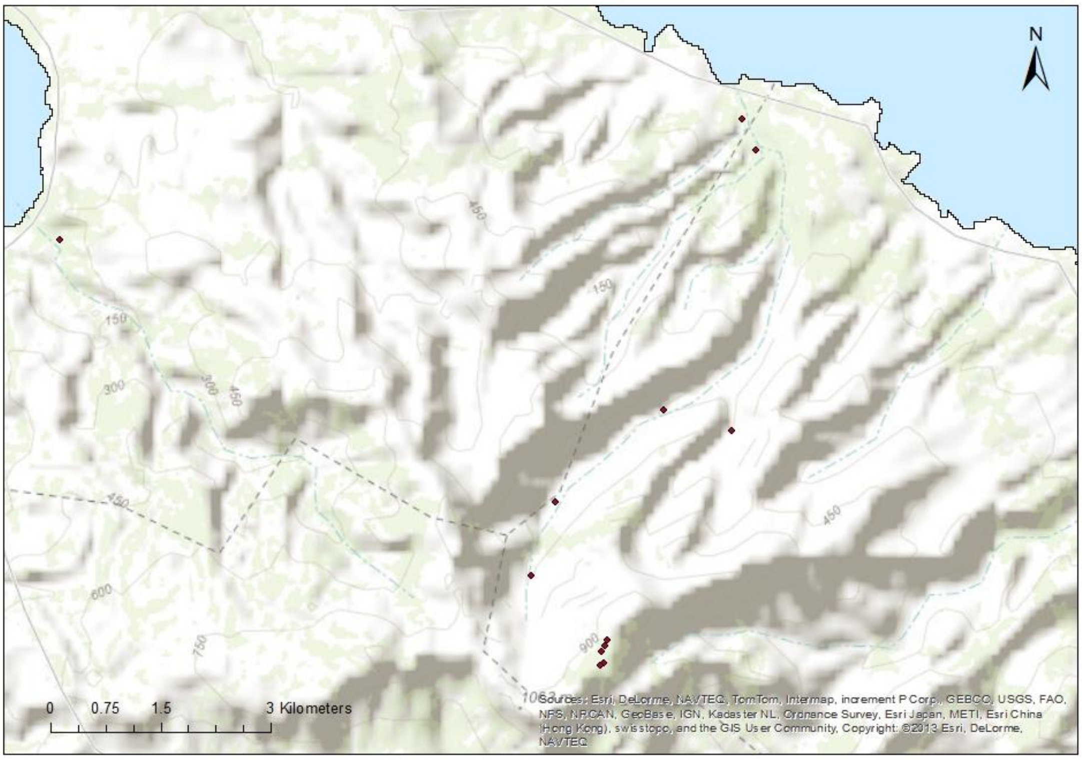
Em Santiago, sendo uma ilha extensa, existe a possibilidade de haver estrutura populacional (i.e., ligeiras diferenças genéticas entre as aves de populações em zonas geográficas distantes). Assim, procurou-se fazer exploração e amostragem de tantos locais quanto possível. Como resultado as aves foram capturadas em três áreas principais: Norte, Centro e Sul (Figure 2 a Figure 5). No Fogo, optou-se por realizar a amostragem junto à localidade de Pai António (Figure 6)), já que esta zona apresenta grande densidade de aves que vivem nas plantações de café e o resto da ilha não apresenta habitat adequado à sobrevivência desta espécie. Em S. Nicolau, procurou-se fazer a amostragem em vários pontos espalhados pela reduzida área que esta espécie ocupa presentemente (Figure 7), tentando amostrar zonas separadas por barreiras geográficas que possam limitar o fluxo genético. Na Brava, cinco locais com potencial habitat adequado foram exaustivamente pesquisados entre 23 a 27 de Janeiro de 2014 (Figure 8). Infelizmente, não foram encontradas felosas de Cabo Verde lá, confirmando assim observações prévias feitas pelo técnico Herculano Dinis (Parque Natural do Fogo) e o naturalista Cornelis Hazevoet (Sociedade Cabo Verdiana de Zoologia). Devido a limitações de tempo, não foram efectuadas buscas em Santo Antão, onde, apesar de não existirem registos desta espécie, parece haver habitat adequado.

> On Santiago, a large island, there may be population structure (i.e., small genetic differences between populations of birds in distant geographical areas). So we tried to explore and sample as many locations as possible. As a result the birds were captured in three main areas: North, Centre and South (Figure 2 to Figure 5). On Fogo, we chose to sample the birds around Pai António (Figure 6), as this area has a high density of birds and the rest of the island does not have suitable habitat for this species. On S. Nicolau, we sampled various locations within the small area that this species presently occupies (Figure 7), trying to include areas separated by geographical barriers that may limit gene flow. On Brava we thoroughly searched five sites with potential suitable habitat from 23rd to 27th January 2014 (Figure 8). Unfortunately, no warblers were found, confirming previous fingings by the technician Herculano Dinis (Fogo Natural Park) and the naturalist Cornelis Hazevoet (Cape Verde Zoological Society). Due to time constraints, no searches were conducted on Santo Antão where, although there are no records of this species, there appears to be suitable habitat.



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community, Copyright: ©2013 Esri, DeLorme, NAVTEQ

Figure 2 – Vista geral da distribuição dos locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis* na Ilha de Santiago / Overview of the sites where Cape Verde warblers were sampled on Santiago.



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community, Copyright: ©2013 Esri, DeLorme, NAVTEQ

Figure 3 - Locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis* na zona Norte da Ilha de Santiago / Sites where Cape Verde warblers were sampled in the North area of Santiago.

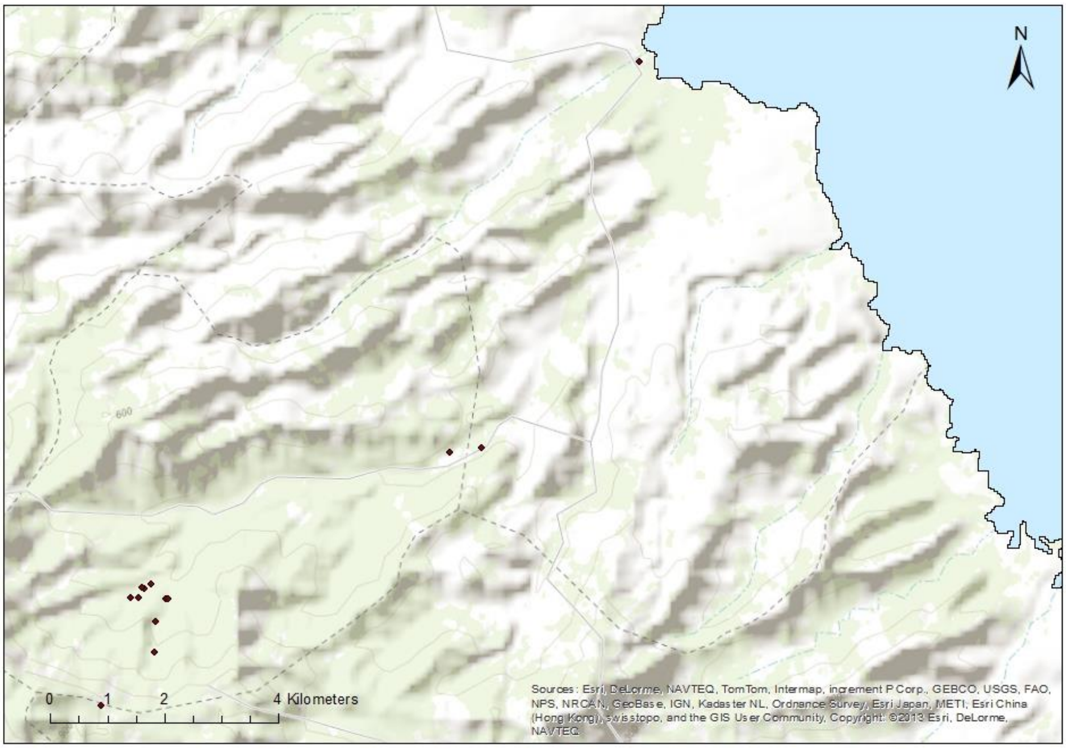


Figure 4 - Locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis* na zona Centro da Ilha de Santiago / Sites where Cape Verde warblers were sampled in the Centre area of Santiago.

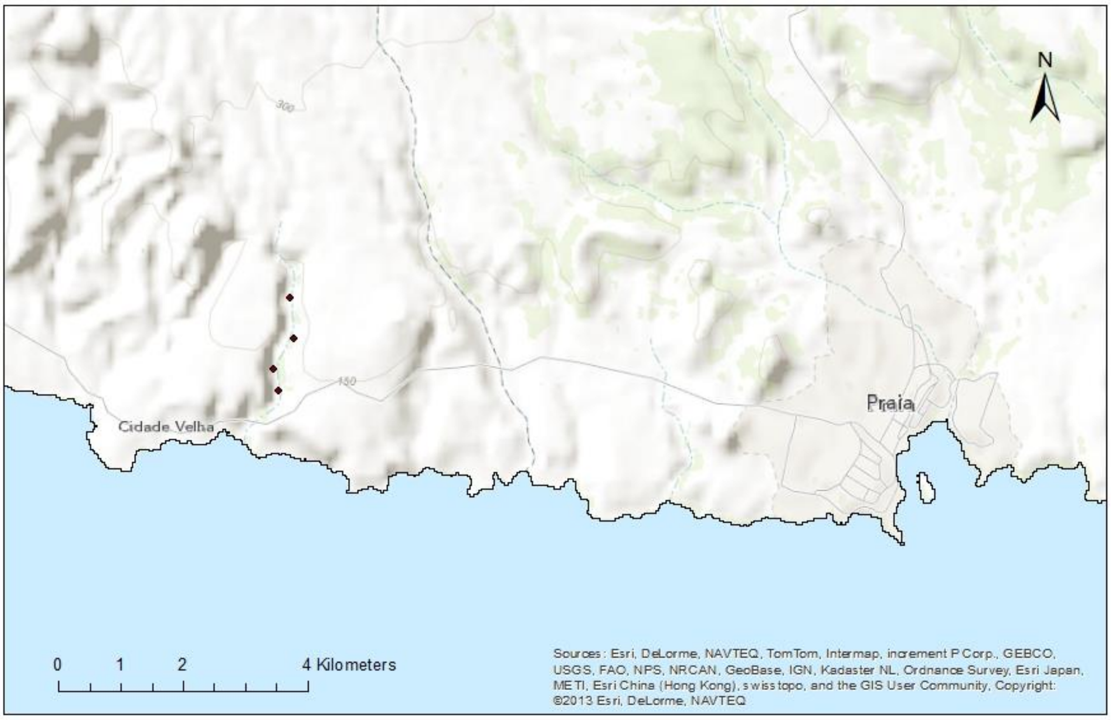
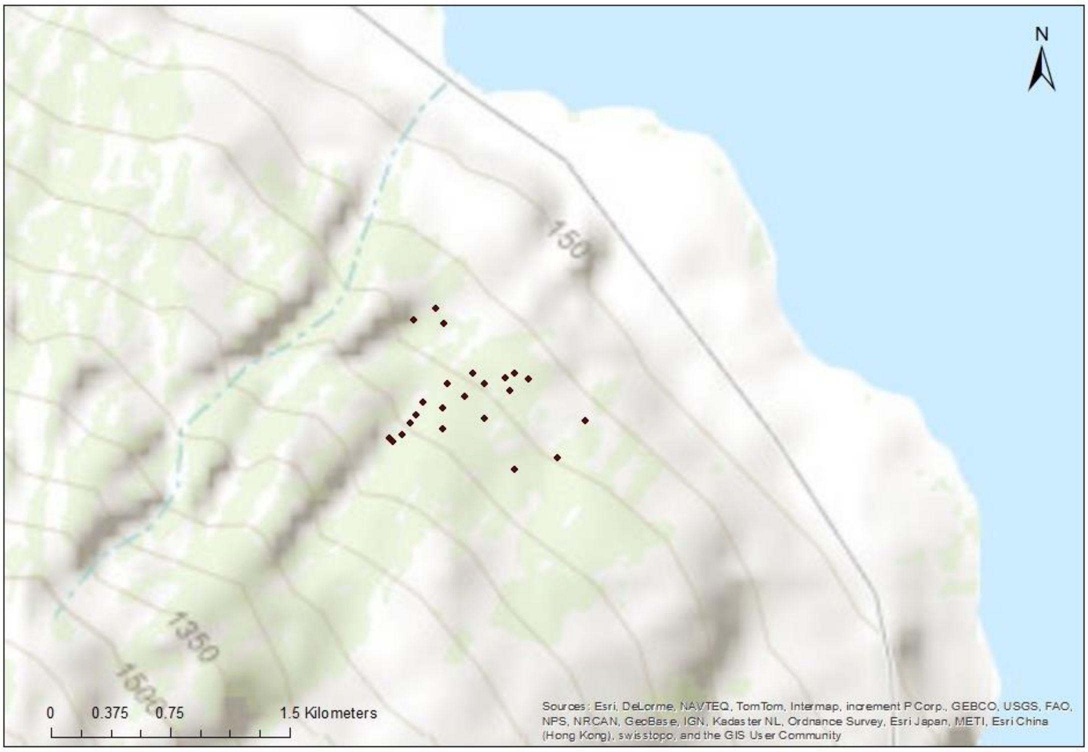
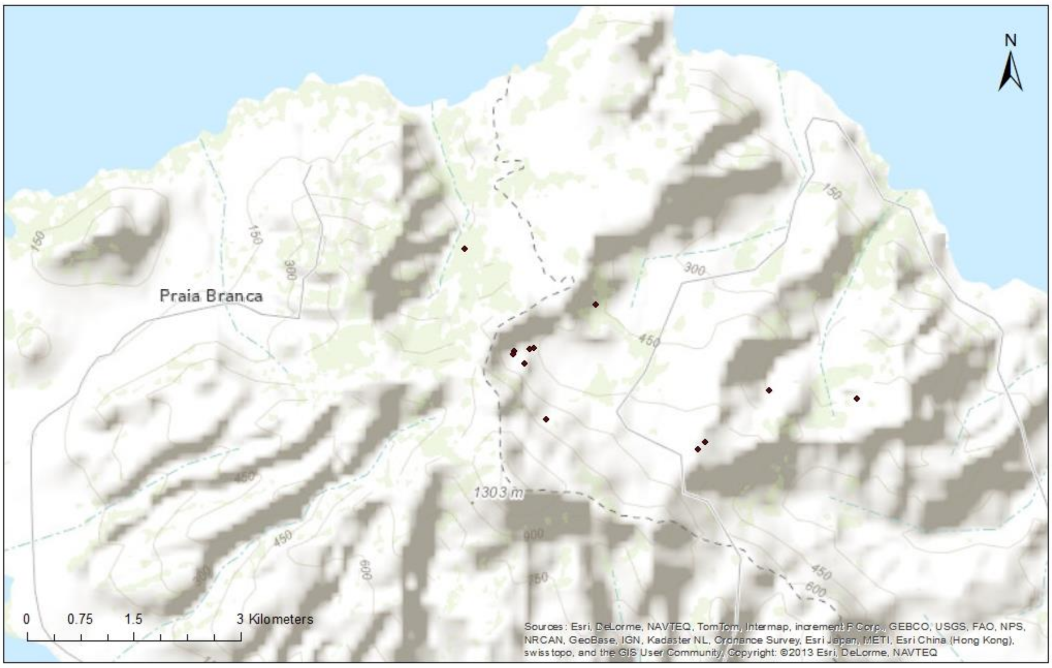


Figure 5 - Locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis* na zona Sul da Ilha de Santiago / Sites where Cape Verde warblers were sampled in the South area of Santiago.



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Figure 6 - Vista da distribuição dos locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis* na Ilha do Fogo / Overview of the sites where Cape Verde warblers were sampled on Fogo.



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community, Copyright: ©2013 Esri, DeLorme, NAVTEQ

Figure 7 - Vista da distribuição dos locais onde foram recolhidas amostras de sangue de felosa de Cabo Verde *Acrocephalus brevipennis* na Ilha de S. Nicolau / Overview of the sites where Cape Verde warblers were sampled on S. Nicolau.

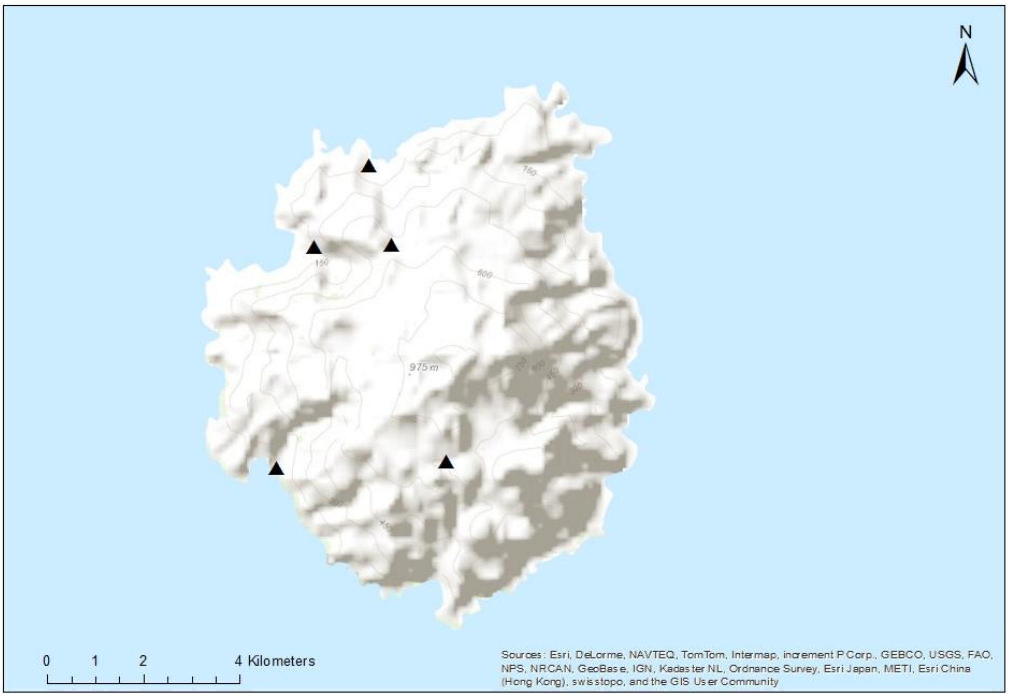


Figure 8 - Locais onde foi procurada a felosa de Cabo Verde *Acrocephalus brevipennis* na Ilha Brava / Sites where we searched for the Cape Verde warbler on Brava.

3.4. Dados morfológicos e fisiológicos | Morphological and physiological data

Foi possível recolher bastantes dados biométricos das felosas de Cabo Verde amostradas (Tables II a V). Além das medidas biométricas, os dados morfológicos incluem o estado físico da ave (massa muscular, gordura). Os dados da muda das penas (estado e progressão) permitiram perceber que nesta espécie os adultos fazem uma muda total das penas após a reprodução. Os dados da reprodução (pelada de incubação) indicam que as aves estavam a terminar, ou tinham já terminado, a sua época de reprodução na altura do ano em que foram amostradas. Foram ainda recolhidos dados de infestação por parasitas, avaliando a quantidade de ectoparasitas nas penas de vôo e recolhendo amostras de fezes quando possível. No total foram recolhidas 34 amostras de fezes, 16 em aves de Santiago, 14 no Fogo e quatro em S. Nicolau. Estes dados serão conjugados com dados genéticos de modo a avaliar a capacidade do sistema imunitário destas aves. Os métodos de recolha destas medidas estão descritos no Anexo I e os dados recolhidos para cada ave capturada estão disponíveis nas tabelas XI e XII do Anexo II.

> Standard biometric measurements were taken from every bird caught (summaries on Tables II to V) including measures to capture the physical condition of the bird (muscle, fat). The data on moult (state and progression) showed that in this species the adults make a complete moult after the breeding season. Brood patch data indicate that birds had finished breeding at the time of the year

in which they were sampled. Parasite data were also collected, by counting the number of mites on flight feathers and collecting faecal samples when possible. We collected 34 faecal samples, 16 on Santiago, 14 on Fogo and four on S. Nicolau. Parasite data will be combined with genetic data to assess the health of the birds' immune system. The methods used to collect these measures are described in Appendix I and data collected for each bird captured are available in tables XI and XII in Appendix II.

Table II – Sumário dos dados morfológicos de felosa de Cabo Verde *Acrocephalus brevipennis*, recolhidos em cada ilha e agrupado por sexo; em cada coluna apresentam-se os valores médios, e entre parêntesis o desvio padrão; F = fêmea; M = macho; N = total / Summary of Cape Verde warbler biometrics, collected on each island and grouped by bird sex; on each cell, the average is followed by the standard deviation, between brackets; F = female; M = male; N = total.

	Média por sexo e por ilha / Average by sex and island (Desvio padrão / Standard deviation)							
Medida / Measurement	Comprimento da asa / Wing length	Comprimento do tarso / Tarsus length	Comprimento de cabeça e bico / Head plus bill length	Comprimento do bico (até narina) / Bill length (to nostril)	Profundidade do bico / Bill depth	Largura do bico / Bill width	Peso / Weight	**N**
Santiago	**64.0 (2.0)**	**26.7 (1.2)**	**38.5 (1.2)**	**11.2 (0.8)**	**3.5 (0.3)**	**5.3 (0.5)**	**17.2 (1.4)**	**50**
F	62.6 (1.5)	25.7 (0.8)	37.8 (0.9)	11.0 (0.6)	3.5 (0.2)	5.1 (0.6)	16.2 (1.3)	20
M	65.0 (1.6)	27.4 (1.0)	39.0 (1.1)	11.4 (0.9)	3.5 (0.3)	5.3 (0.4)	17.9 (1.0)	30
Fogo	**64.6 (2.1)**	**26.7 (0.9)**	**39.2 (0.9)**	**11.3 (0.5)**	**3.6 (0.2)**	**5.3 (0.2)**	**17.4 (2.1)**	**30**
F	62.4 (1.3)	26.1 (0.9)	38.3 (0.8)	10.8 (0.6)	3.6 (0.2)	5.2 (0.2)	16.6 (1.2)	8
M	65.4 (1.70)	27 (0.9)	39.5 (0.6)	11.5 (0.4)	3.7 (0.1)	5.4 (0.2)	17.8 (2.2)	22
S. Nicolau	**64.4 (1.3)**	**26.0 (0.9)**	**39.8 (0.9)**	**12 (0.7)**	**3.6 (0.1)**	**5.3 (0.2)**	**18.1 (1.6)**	**15**
F	63 (0.0)	24.5 (0.4)	38.7 (1.0)	11.1 (1.0)	3.4 (0.1)	5.2 (0.1)	16.5 (0.9)	3
M	64.8 (1.2)	26.4 (0.6)	40.1 (0.6)	12.2 (0.4)	3.6 (0.1)	5.4 (0.2)	18.6 (1.4)	12
Média geral / Population average	**64.3 (1.9)**	**26.6 (1.1)**	**38.9 (1.2)**	**11.4 (0.7)**	**3.6 (0.2)**	**5.3 (0.4)**	**17.4 (1.7)**	**95**

Table III – Sumário das classificações de gordura (colunas) e músculo (linhas) de felosa de Cabo Verde *Acrocephalus brevipennis*; gordura: 0 = ausente; 1 = muito pouca; 2 = pouca; músculo: 2 = normal; 3 = bem constituído; para descrição compreensiva das classificações ver Anexo I / Summary of Cape Verde warbler fat (columns) and muscle (rows) scores; fat scores: 0 = absent; 1 = very little; 2= little; muscle: 2 = normal; 3 = well developed; for full description of scores see Appendix I.

	Gordura / Fat score			
Músculo / Muscle score	0	1	2	**Total**
Santiago	**30**	**12**	**5**	**47**
2	16	4	2	22
3	14	8	3	25
Fogo	**22**	**5**	**3**	**30**
2	17	2	2	21
3	5	3	1	9
S. Nicolau	**10**	**5**	**0**	**15**
2	6	4	0	10
3	4	1	0	5
Total	**62**	**22**	**8**	**92**

Table IV - Sumário das classificações de muda e pelada de incubação (colunas), por sexo (linhas) de felosa de Cabo Verde *Acrocephalus brevipennis*. Muda: O = inactiva; S = a iniciar; B = muda das penas corporais; M = muda das penas de voo; E = terminada. Pelada de incubação: 0 = ausente; 2 = em desenvolvimento; 3 = estádio máximo; 4 = a regredir; 5 = a fechar; para descrição compreensiva das classificações ver Anexo I. F = fêmea; M = macho; N = total. / Summary of Cape Verde warbler moult and brood patch (columns) scores, by sex (rows); Moult: O = inactive; S = starting; B = body feathers moulting; M = main moult; E = ending. Brood patch: 0 = absent; 2 = developing; 3 = full brood patch; 4 = regressing; 5 = closing; for full description of scores see Appendix I. F = female; M = male; N = total.

	Muda / Moult											
	O				**S**		**B**	**M**			**E**	
Pelada de incubação / Brood patch	0	2	3	4	0	4	0	0	4	5	0	**N**
Santiago 18/11/2013 - 14/12/2013	**20**	**1**	**2**	**3**	**3**	**1**	**0**	**14**	**0**	**3**	**0**	**47**
F	2	1	2	3	0	1	0	6	0	2	0	17
M	18	0	0	0	3	0	0	8	0	1	0	30
Fogo 19/12/2013 - 31/12/2013	**1**	**0**	**0**	**1**	**0**	**0**	**1**	**15**	**1**	**10**	**0**	**29**
F	1	0	0	1	0	0	0	3	1	2	0	8
M	0	0	0	0	0	0	1	12	0	8	0	21
S. Nicolau 04/01/2014 - 19/01/2014	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**11**	**0**	**0**	**2**	**15**
F	0	0	0	0	0	0	0	2	0	0	1	3
M	1	0	0	0	1	0	0	9	0	0	1	12
Total	**22**	**1**	**2**	**4**	**4**	**1**	**1**	**40**	**1**	**13**	**2**	**91**

Table V - Sumário das classificações da contagem de parasitas das penas na felosa de Cabo Verde *Acrocephalus brevipennis*; A = 0; B = 1; C = 2-5; D = 6-10; E = 11-20; F = 21-100; G = >100; para descrição compreensiva das classificações ver Anexo I / Summary of Cape Verde warbler feather mite score; A = 0; B = 1; C = 2-5; D = 6-10; E = 11-20; F = 21-100; G = >100; for full description of scores see Appendix I.

Parasitas das penas / Feather mites	A	B	C	D	E	F	G	**Total**
Santiago	7	1	1	4	3	23	1	**40**
Fogo	0	0	0	2	5	23	0	**30**
S. Nicolau	4	0	1	0	3	7	0	**15**
Total	**11**	**1**	**2**	**6**	**11**	**53**	**1**	**85**

3.5. Canto | Song

Foram registados um total de 123 ficheiros de som da felosa de Cabo Verde, nas três ilhas. Destes, 87 foram registados em Santiago, 17 em Fogo e 19 em S. Nicolau. Esses arquivos correspondem a um mínimo de 55 machos individuais (40 de Santiago, 11 de Fogo e 4 de S. Nicolau). Os machos de Santiago pareceram reagir a um canto gravado em Assomada, Santiago, em 2005, por Antero Lindholm (disponível em http://www.xeno-canto.org/), sendo atraídos para as redes por ele. Os machos de Fogo também reagiram a este canto, mas pareceram reagir mais fortemente a uma das primeiras gravações de boa qualidade registadas no Fogo. Da mesma forma, os machos de S. Nicolau pareceram reagir mais fortemente a uma gravação feita nessa ilha que às gravações feitas em Fogo ou Santiago. Note-se, no entanto, que estas reacções não foram avaliadas quantitativamente, e que estas avaliações são subjectivas.

> We recorded a total of 123 sound files at 56 different locations across the islands; 87 in Santiago, 17 in Fogo and 19 in S. Nicolau. These files correspond to at least 55 Cape Verde warbler males (40 from Santiago, 11 from Fogo and 4 from S. Nicolau). Santiago males reacted to a song recorded in Assomada, Santiago, in 2005, by Antero Lindholm (available at http://www.xeno-canto.org/), being attracted to the nets when we played it. The males of Fogo also reacted to this Santiago song, but appeared to react more strongly to one of the first recordings of good quality made on Fogo. Likewise, S. Nicolau males appeared to react more strongly to a recording made on this island that to the recordings made in Fogo or Santiago. However, it must be noted that these reactions were not quanitatively assessed and therefore they are just subjective observations.

3.6. Dados de habitat | Habitat data

Nesta primeira época de campo, foi dada prioridade à recolha de amostras de sangue das aves. Foram recolhidos alguns dados de habitat mas não de forma sistemática. A composição do habitat da felosa de Cabo Verde parece ser muito diferente, tanto entre as ilhas como dentro de cada ilha, tanto em espécies de plantas como em variáveis abióticas como altitude, temperaturas médias, nebulosidade. Foi feita uma avaliação preliminar destas características, que servirá como base para a concepção de um modelo adequado para a próxima época de campo. Nesta avaliação preliminar simplificada foram registados apenas: (1) a presença ou ausência de árvores, água e agricultura, (2) o tipo de terreno (vale ou encosta), (3) a percentagem de área coberta de vegetação rasteira e percentagem de área coberta de sombra e (4) uma descrição sumária do tipo de vegetação encontrada. Todos os registos e estimativas foram feitos, muito grosseiramente, apenas na área que as felosas foram observadas a utilizar.

Não foi possível estimar o tamanho do território utilizado pelas aves, mas quaisquer re-avistamentos ou recapturas de aves anilhadas com anilhas de cor foram registadas.

> During this field season we prioritized collecting blood samples from birds so although some habitat data was collected, this was not done systematically. The composition of the Cape Verde warbler habitat seems to be very different between and within the islands, both in terms of plant species and in abiotic variables such as altitude, average temperatures, or cloudiness. We did a preliminary evaluation of these features, which will serve as the basis for designing an appropriate protocol for the next field season. We noted (1) the presence or absence of trees, water and agriculture, (2) the type of terrain (valley bottom or slope), (3) the percentage of area covered with undergrowth and percentage of shaded area and (4) a summary of the type of vegetation found. All estimates were made only in the area that the birds were seen using. It was not possible to estimate any territory sizes but all re-sightings and recaptures of colour-ringed birds were recorded.

3.7. Métodos de censo | Censusing methods

Durante esta primeira época de campo, foram registadas com Global Positioning System (GPS), modelo Garmin eTrex® H, todas as aves detectadas por observação ou som (com ou sem recurso a playback) no decorrer das saídas de campo, todos os ninhos identificados e todos os locais explorados em que as aves não foram observadas (os chamados *pontos negativos*). A maior parte dos ninhos encontrados foram identificados por Gilson Semedo (INIDA, Santiago) ou pelos colaboradores de Pai António (Fogo). Ao longo da época de campo, foi sendo avaliada de forma informal a eficiência do uso de transectos e pontos de escuta, com ou sem uso de playbacks, na detecção da felosa de Cabo Verde (Bibby *et al.* 1992; Showler *et al.* 2002). Na ilha do Fogo não foram realizados censos de teste fora da área nordeste, que tem vegetação densa e plantações de café. Fora desta área não existe habitat adequado para esta espécie, e portanto não foram registados pontos negativos para esta ilha.

> During this first field season we used Global Positioning System (GPS) Garmin eTrex® H to record the location of all birds detected by sight or sound, all nests identified and all the explored sites where birds were not observed (negative points). Most nests were identified by Gilson Semedo (INIDA, Santiago) or by inhabitants of Pai António (Fogo). Throughout the field season we assessed in an informal way the efficiency of transects and point counts, with or without the use of playback, to detect the Cape Verde warbler (Bibby *et al.* 1992; Showler *et al.* 2002). On Fogo, no trial censuses were conducted outside the northeastern areas, with dense vegetation and coffee plantations. Outisde this area there is no suitable habitat and no Cape Verde warblers, hence no negative points were recorded on this island.

4. Conclusões | Conclusions

Concluímos que esta primeira época de campo teve excelentes resultados, tendo sido atingidos todos os principais objectivos propostos.

The first field season of this project had excellent results and all key objectives were achieved.

1. Foram iniciadas colaborações sólidas com todas as entidades nacionais envolvidas na conservação da felosa de Cabo Verde (DGA, INIDA e Parques Naturais da Serra Malagueta, Fogo e Monte Gordo);

   - We initiated close collaborations with all national authorities involved in the conservation of the Cape Verde warbler (DGA, INIDA and Natural Parks of Serra Malagueta, Fogo and Monte Gordo);

2. Foi dada formação inicial em anilhagem de aves a técnicos de todas estas entidades;

   - Initial training in bird ringing was given to technicians from all these authorities;

3. Obteve-se o número mínimo de 30 amostras de sangue de felosa de Cabo Verde nas ilhas de Santiago e Fogo, e 15 amostras em S. Nicolau;

   - We obtained the minimum number of 30 blood samples from the Cape Verde Warbler on the islands of Santiago and Fogo, and 15 samples on S. Nicolau;

4. Foram recolhidos parâmetros adicionais morfológicos e fisiológicos de todas as aves amostradas;

   - Additional morphological and physiological parameters were recorded for all birds sampled;

5. Foram gravados cantos de um mínimo de 10 machos em Santiago e Fogo, e 4 em S. Nicolau;

   - We recorded songs of a minimum of 10 males on Santiago and Fogo, and 4 on S. Nicolau;

6. Foram recolhidos dados de base sobre as características do habitat utilizado pela espécie em todas as ilhas;

   - We collected baseline data on habitat features on all islands;

7. Foram testados vários métodos de censos de aves e várias adaptações dos mesmos ao caso da felosa de Cabo Verde.

   - We tested various methods of bird censuses and adaptations of these methods to the case of the Cape Verde warbler.

5. Discussão | Discussion

5.1. Colaboração com entidades nacionais | Collaboration with national stakeholders

Considera-se que o objectivo de iniciar colaborações com entidades nacionais envolvidas na conservação da felosa de Cabo Verde foi atingido. Tivemos oportunidade de trabalhar com todas as entidades que monitorizam esta espécie, em todas as ilhas onde a mesma ocorre. A troca de informações entre a equipa técnica e os parceiros Cabo Verdeanos foi excelente, facilitando a aprendizagem de ambas as partes e contribuindo efectivamente para a recolha de dados de qualidade no campo.

> The aim of initiating collaborations with national organizations involved in the conservation of the Cape Verde Warbler was achieved. We had the opportunity to work with all the stakeholders that monitor this species on all the islands where it occurs. The exchange of information between the technical team and Cape Verde partners was excellent, facilitating learning for both parties and effectively contributing to the collection of quality data in the field.

5.2. Treino de técnicos locais | Training of local technicians

Considera-se que o objectivo de dar formação aos técnicos locais para continuar a monitorização da espécie foi atingido. Os técnicos que tiveram formação em anilhagem podem futuramente seguir as aves marcadas com anilhas de cor e assim estudar o território que as mesmas utilizam. Porém, algumas saídas de campo não são suficientes para dotar os técnicos de uma formação sólida que lhes permita efectuar este tipo de trabalho de forma independente no futuro.

> The aim of training local technicians in ringing methods to continue monitoring the species in the future was achieved. The technicians trained can now follow the birds marked with colour rings and study their territory use. However, the limited amount of field trips and ringing training given so far was not enough to provide sufficient training to enable them to carry out ringing work independently in the future.

5.3. Amostras de sangue | Blood samples

O objectivo de recolher um mínimo de 30 amostras de sangue em cada ilha foi atingido em Fogo (30), ultrapassado em Santiago (50) e não atingido em S. Nicolau (15). Idealmente, para se obterem resultados válidos, as 30 amostras mínimas devem pertencer a indivíduos não relacionados, para não haver sobre-representação dos mesmos alelos. Na prática, isso significa que em cada território os juvenis só contam como amostras independentes se os adultos não tiverem sido amostrados. Ou seja, se recolhermos amostras de um adulto e um juvenil do mesmo território, na realidade só temos uma amostra válida para esse território. Na ilha do Fogo, foram amostrados 3 juvenis de territórios onde já tinham sido amostrados adultos. Embora todos os dados sejam úteis, isto significa que só temos 27 amostras válidas da população do Fogo, número ligeiramente abaixo do recomendado. Do mesmo modo, como a população de felosa de Cabo Verde na ilha de S. Nicolau é reduzida e difícil de amostrar, não foi possível conseguir as 30 amostras no tempo disponível. Por outro lado, em ilhas extensas como Santiago, em que pode existir estrutura populacional entre várias localidades da ilha, é recomendável obter um mínimo de 30 amostras de cada área estudada (Norte, Centro e Sul). Apesar de não ser dada prioridade, neste momento, à caracterização da estrutura populacional intra-insular, aproveitou-se o facto de termos que recolher amostras em várias localidades para recolher o máximo possível, tendo em vista a possibilidade de estudar diferenças entre as populações de Santiago numa segunda fase deste projecto.

> The collection of 30 blood samples was achieved on Fogo (30) and surpassed in Santiago (50) but only 15 were collected in S. Nicolau. To obtain valid results, the 30 samples should belong to unrelated individuals, to avoid over-representation of similar alleles. In practical terms, this means that in each territory juveniles only count as independent samples if adults have not been sampled there. During this season, three juveniles were sampled from territories where adults had already been sampled on Fogo. Thus we have only 27 valid samples of the Fogo population. Similarly, as the population of Cape Verde warbler of the island of S. Nicolau is very small and difficult to sample, we could not get the 30 samples in the time available. Furthermore, on large islands like Santiago, where population structure may exist, it is recommended to obtain a minimum of 30 samples from each study area (North, Centre and South). On Santiago, we took advantage of the fact that we had to collect samples at various locations to collect as many as possible, foreseeing the possibility of studying differences among populations within this island in a second phase of this project.

5.4. Dados morfológicos e fisiológicos | Morphological and physiological data

Em geral, as medidas biométricas das aves amostradas parecem corresponder ao que se esperava com base em medições anteriormente descritas na literatura (Dohrn 1871), ou recolhidos em espécimes de museu (N. Collar, pers. comm.). Outras medidas fisiológicas (por exemplo, muda, pelada de incubação) também corresponderam ao que seria de esperar tendo em conta que esta é uma espécie residente que se reproduz durante a época das chuvas (Bourne de 1955; Hazevoet 1995). Até agora, os machos com pelada de incubação só foram encontrados na população de Fogo, mas não está claro se isto é devido a diferenças entre populações ou simplesmente a diferenças no momento da recolha de dados. Além disso, verificámos com surpresa que a maioria das aves amostradas em áreas agrícolas tiveram contagens altas de ácaros de penas. Por outro lado, aves amostradas em áreas de montanha, em altitudes elevadas e com menos contacto com humanos, estavam em geral livre de parasitas. No entanto, o número de aves amostradas em zonas de montanha foi muito pequeno para permitir fazer comparações válidas.

> In general, biometrics of the sampled birds seem to correspond to what was expected based on measurements available in literature (Dohrn 1871) or collected from museum specimens (N. Collar, pers. comm.). Other physical scores (i.e., moult, brood patch) also corresponded to what we would expect considering that this species is a resident bird breeding during the rain season (Bourne 1955; Hazevoet 1995). Until now males with a brood patch were only found in the population of Fogo, but it is unclear whether this is due to differences between populations or simply to differences in timing of data collection. Additionally, we noticed with surprise that the majority of birds sampled in agricultural areas had a high count of feather mites. On the other hand, birds sampled in mountain areas, at high altitudes and with less contact with humans, were generally free of parasites. However, the number of birds sampled in mountain areas was too small to allow making valid comparisons.

5.5. Canto | Song

O objectivo de gravar o canto de um mínimo de 10 machos em cada ilha foi atingido em Santiago e Fogo, mas não atingido em S. Nicolau. Nesta última ilha, foi bastante difícil conseguir gravar o canto dos machos porque (1) as aves são difíceis de encontrar e (2) vocalizam pouco. A reacção das aves em S. Nicolau foi muito fraca: por vezes, algumas nem sequer vocalizavam em resposta à gravação, apesar de estarem presentes no território (confirmado por observação ou captura). Não é claro se isso se deve: (1) ao facto da amostragem em S. Nicolau ter sido feita após o final da época de reprodução; (2) a diferenças de comportamento nas aves desta população; (3) ou a uma combinação dos dois factores.

> We recorded 10 males on Santiago and Fogo, but not on S. Nicolau. On the latter island it was quite difficult to record male songs because (1) the birds were hard to find and (2) they vocalized little. The reaction of the birds on S. Nicolau was very weak: it happened that the birds did not react to the playback, despite being present in the territory (as confirmed by observations or captures). It is unclear whether this is due to: (1) the fact that the sampling on S. Nicolau was done after the end of

> the breeding season; (2) differences in the behaviour of birds of this population; (3) a combination of two factors.

5.6. Dados de habitat | Habitat data

Nesta primeira época de campo, o objectivo de recolher dados de habitat de forma sistematizada não foi atingido. Não foi possível estimar o tamanho do território utilizado pelas aves, para iniciar uma estimativa de uso de espaço / habitat. Testámos o protocolo utilizado para recolher dados de habitat da felosa das Seychelles (Seychelles Warbler Project 2014) mas concluímos não é adequado para a felosa de Cabo Verde, por duas razões. Primeiro, porque esta espécie habita zonas de vegetação arbustiva muito densa e espinhosa, de muito difícil acesso, onde um seguimento continuado da ave pelo território fica muitas vezes impossibilitado. Segundo, porque ao contrário da felosa das Seychelles, esta espécie habita locais bastante diversos em termos de composição da vegetação, não só entre as ilhas mas também dentro de cada ilha. Neste sentido, estamos a desenvolver um protocolo que seja possível de executar no campo e tenha em conta não só toda a diversidade de habitats que a felosa de Cabo Verde usa, procurando avaliar quais as características comuns a todos esses tipos de habitat, mas também que seja rápido e fácil de usar. Um protocolo deste tipo será muito importante para recolher dados que permitam prever a potencial distribuição da espécie.

> In this first field season, we were unable to collect habitat data in a systematic way. It was not possible to estimate territory size used by the birds to initiate an assessment of space use. We tested the protocol used for the Seychelles warbler (Seychelles Warbler Project 2014) but concluded it is not appropriate for the Cape Verde warbler, for two reasons. First, because this species inhabits areas of dense shrubby and thorny vegetation, very difficult to access, where a continuous monitoring of the bird through the territory is often impossible. Second, because unlike the Seychelles warbler, this species inhabits very different places in terms of vegetation composition, not only between the islands but also within each island. Therefore we are developing a protocol that will take into account not only the diversity of habitats that the Cape Verde warbler uses, trying to assess common features, while being efficient use. Such a protocol is important to collect data for predicting the potential distribution of the species.

5.7. Métodos de censo | Censusing methods

Apesar de não terem sido realizados censos formais, a nossa equipa tem agora informações suficientes para realizar censos completos das ilhas, tendo encontrado métodos de censos robustos adaptados à detectabilidade das aves nas diferentes populações. As felosas de Cabo Verde são detectadas mais facilmente pelo som do que por observação, já que usam um habitat com vegetação arbustiva densa. Estas aves não são fáceis de observar e passam bastante despercebidas, por vezes mesmo após longos períodos de escuta. São muito mais conspícuas em Santiago e Fogo do que em S. Nicolau. Por exemplo, nas duas primeiras ilhas reagiram fortemente às gravações, e cantavam ou faziam sons de alarme espontanea e frequentemente, ao passo que em S. Nicolau eram bastante silenciosas e nem sempre reagiram às gravações. Não é claro se esta diferença se deve a diferenças na época de reprodução, diferenças na densidade populacional ou a diferenças comportamentais intrínsecas entre as populações.

É possível que os números reais dos individuos indicados no passado tenham sido subestimados (em Santiago e S. Nicolau) e sobre-estimados (no Fogo). Em Santiago e S. Nicolau encontramos mais aves do que o esperado com base na literatura publicada (Donald *et al.* 2004; N. Wilkinson, pers. comm.; Hazevoet 1995; Hazevoet *et al.* 1999), por diferentes razões: (1) em Santiago, porque procurámos as aves em lugares previamente não explorados (CJ Hazevoet, pers. comm.) e, (2) em S. Nicolau, porque fizemos uma exploração muito minuciosa recorrendo ao uso intensivo de playbacks. Pelo contrário, é possível que a população em Fogo tenha sido anteriormente sobre-estimada. Após anilhar as aves, observámos que elas parecem usar uma área maior do que anteriormente sugerido (Hering & Fuchs 2009; Hering & Hering 2005). É possível que nos estudos anteriores a mesma ave tenha sido observada em vários locais diferentes e erradamente registada como várias aves, levando a uma estimativa exagerada do número de indíviduos. Isto realça a importância de marcar as aves com anilhas coloridas para obter estimativas correctas do número de indivíduos e tamanho do seu território.

Foi possível compreender que as metodologias para fazer censos terão de ser adaptadas a esta espécie, e mais especificamente às diferentes populações, pelo menos se os censos forem realizados após a época de reprodução. Desaconselhamos o uso de métodos de registo de observações visuais ao longo dum transecto, principalmente em S. Nicolau. Um censo completo deverá incluir identificação de sons (canto dos machos, chamamentos de fêmeas ou juvenis, e alarmes), e uso de gravações para obter respostas dos machos. Recomenda-se que os censos sejam feitos por observadores experientes que estejam familiarizados com a espécie. Adicionalmente, observámos que o comportamento das aves e as respostas às gravações variam bastante entre as ilhas. Em Santiago e Fogo, os machos são vocalmente bastante activos e reagem rapidamente às gravações, enquanto que em S. Nicolau são muito silenciosos e pouco reactivos.

> Although no formal censuses had been carried out, we have now sufficient information to design a program to undertake a complete census, using robust methods adapted to the detectability of birds in different populations. The Cape Verde warblers are more easily detected by sound than by sight,

because they use dense shrubby vegetation. These birds are not easy to observe, sometimes even after long searching periods. They are much more conspicuous on Santiago and Fogo than on S. Nicolau. For example, on the first two islands they reacted strongly to playbacks, and sang or made alarm sounds often and spontaneously, whereas on S. Nicolau they were quite silent and not always responded to playbacks. It is unclear whether this is due to differences in the timing of the breeding season, different population densities or intrinsic behavioural differences between populations.

It is possible that the actual numbers of individuals have been underestimated in the past (on Santiago and São Nicolau) and over-estimated (on Fogo). On Santiago and São Nicolau we sampled more birds than expected based on the published literature (Donald *et al.* 2004; N. Wilkinson, pers. comm.; Hazevoet 1995; Hazevoet *et al.* 1999), for different reasons. On Santiago, because we looked for the birds in places previously unexplored (CJ Hazevoet, pers. comm.), and on São Nicolau because we did a thorough exploration with intensive use of playbacks. By contrast, it is possible that the population on Fogo has been previously over-estimated. After colour-ringing the birds, we noticed that individuals seem to use larger areas than previously suggested (Hering & Fuchs 2009; Hering & Hering 2005). It is possible that in previous studies, the same bird has been observed in several different locations and erroneously recorded as several different birds. This highlights the importance of colour-ringing the birds for correct estimates of the number of individuals and territory sizes.

We concluded that the methodologies for conducting censuses need to be adapted to the species, and more specifically to different populations, at least if the census is conducted after the breeding season. We discourage using methods that rely on visual observations along transects, especially on S. Nicolau. A complete census should include identification of sounds (male song, females and juveniles calls, and alarm sounds), and use playbacks to trigger male replies. We recommend that censuses are made by experienced observers who are familiar with the species and its sounds. Additionally, we noticed that the birds' behavior and responses to playbacks seem to vary greatly between islands. On Santiago and Fogo, males are vocally very active and react quickly to playbacks, whereas in S. Nicolau they are very quiet and not very reactive.

6. Direcções futuras | Future directions

Dado que a primeira época de campo deste projecto teve resultados bastante positivos, recomendamos a continuação e desenvolvimento do mesmo nos próximos anos. É importante recolher mais dados sobre o canto, características do habitat e tamanho das populações de felosa de Cabo Verde, pois isso fornece informação essencial para conhecer a espécie e implementar futuras medidas de conservação de sucesso. Propomos realizar mais épocas de campo mantendo a colaboração já iniciada entre a Universidade de East Anglia e as entidades Cabo Verdeanas.

> As the first field season was successful we strongly recommend the continuation and development of this project in the next years. It is important to collect more data on song, habitat features and population sizes, as this will inform future conservation measures and determine their success. We aim to conduct more field seasons in the future and maintain the collaboration initiated between the University of East Anglia and the Cape Verdean stakeholders.

6.1. Colaboração com entidades nacionais | Collaboration with national stakeholders

É do interesse do projecto e de todos os parceiros que as colaborações iniciadas se mantenham no futuro. Estas colaborações irão facilitar a transmissão de experiências e conhecimentos entre a Universidade de East Anglia e as entidades locais. Isto permite, por um lado, aumentar a qualidade dos dados recolhidos pela equipa no âmbito deste projecto e, por outro lado, que os técnicos locais acompanhem o processo em primeira mão, contribuindo activamente para o desenvolvimento e implementação dos protocolos. Nesse sentido, sugerimos que seja mantido o contacto com todas as entidades envolvidas na monitorização da felosa de Cabo Verde.

> For the success of this project, it is crucial that all initiated collaborations are maintained in the future. These collaborations will facilitate experience and knowledge exchange between the University of East Anglia and local stakeholders. This allows to increase the quality of data collected by the team and, moreover, for local technicians to monitor the process, actively contributing to the development and implementation of protocols. Accordingly, we suggest that contact with all entities involved in monitoring the Cape Verde warbler is maintained.

6.2. Treino de técnicos locais | Training of local technicians

Aconselhamos que os técnicos locais devem continuar a sua formação em metodologias de monitorização de fauna que envolvam marcação e recaptura. Os técnicos demonstraram, na primeira época de campo, grande interesse, capacidade de aprendizagem e sólidos conhecimentos de base sobre a ecologia das aves, pelo que é aconselhável que continuem a desenvolver as suas competências técnicas. Isto permitirá que sejam capazes de realizar trabalhos de marcação e seguimento de aves com anilhas de forma independente no futuro. Neste sentido, propomos continuar a trabalhar conjuntamente com todos os interessados, continuando assim a partilhar conhecimentos práticos e teóricos sobre o uso da anilhagem na monitorização de aves selvagens.

> We recommend that local technicians should continue their training in capture-mark-recapture wildlife monitoring techniques. During the first field season, the technicians we worked with showed enthusiasm and ability and had solid basic knowledge about the ecology of birds. If they continue to develop their technical skills, this will ultimately allow them to mark and monitor ringed birds independently in the future. Therefore, we propose to continue working together with all stakeholders, continuing to share practical and theoretical knowledge on the use of ringing in the monitoring of wild birds.

6.3. Amostras de sangue | Blood samples

Tendo em conta que o número mínimo de amostras aconselhado não foi conseguido em S. Nicolau, recomendamos regressar a S. Nicolau para amostrar mais aves. O número efectivo de amostras ficou ligeiramente abaixo do recomendado na ilha do Fogo, e não foi possível obter amostras de indivíduos residentes na montanha (Monte Velha), pelo que recomendamos regressar a esta ilha e tentar amostrar aves nessa área. Similarmente, se forem detectados indícios de estrutura populacional entre as zonas Norte, Centro e Sul de Santiago nas análises genéticas, é aconselhavel recolher mais amostras em cada uma destas zonas para validar os resultados. Em todo o caso, e uma vez que o objectivo deste projecto é monitorizar a espécie a longo prazo, recomenda-se que vão sendo recolhidas mais amostras em cada ilha, sobretudo de populações ainda não amostradas, para seguir a evolução da diversidade genética das populações ao longo do tempo. Adicionalmente, consideramos desejável continuar a anilhar e medir aves de outras espécies que caiam acidentalmente nas redes, uma vez que isto possibilita a recolha de bastantes dados de várias espécies com um acréscimo mínimo de custo e esforço para o nosso projecto. Concordamos em seguir as recomendações da DGA e INIDA de respeitar o período de reprodução da espécie, recolhendo amostras apenas antes ou depois desta época, para não perturbar as aves.

> As we could not collect the recommended minimum number of sample in S. Nicolau, we recommend returning there to sample more birds. The actual number of samples was also slightly lower than recommended on Fogo, and it was not possible to obtain samples from individuals living in the

mountain (Monte Velha), so we also recommend returning to this island and try to sample birds in this area. Similarly, if we find evidence of population structure within Santiago, it is advisable to collect more samples in each of the surveyed areas to validate the results. In any case, since the goal of this project is to monitor the long-term genetic diversity of this warbler, it is recommended that more samples are collected on each island, from sites not yet sampled. Additionally, we propose to continue ringing and measuring other species that are caught on the nets by accident, since this allows data collection from several species with a minimum increase in cost and effort to our project. We agree to follow the recommendations of DGA and INIDA regarding disturbance avoidance during the breeding period of the species by sampling just before or after this time.

6.4. Dados morfológicos e fisiológicos | Morphological and physiological data

É necessário clarificar se existem diferenças entre as populações em termos de época de reprodução e progressão da muda. É importante, portanto, regressar às várias ilhas e anilhar e medir as aves em alturas desfasadas do ano anterior. É igualmente necessário clarificar se as aves de zonas agrícolas e de montanha têm diferentes graus ou tipos de parasitas, de modo a (1) poder avaliar o risco a que estão expostas, que pode influenciar a necessidade de medidas de conservação diferentes, e (2) compreender se as aves nestas zonas estão adaptadas a estas forças selectivas. Assim, propomos regressar a Cabo Verde no final de Outubro / ínicio de Novembro, iniciando a amostragem em S. Nicolau ou no Fogo, prosseguindo por fim para Santiago, terminando a amostragem no final de Dezembro, insistindo na amostragem de aves de zonas de montanha (Monte Velha, Monte Tchota, Serra Malagueta).

It is important to clarify whether there are differences between the populations in the timing of breeding season and moult. It is important, therefore, to return to Cape Verde and ring and measure the birds at different times from the previous year. It is also necessary to clarify whether the birds in agricultural and mountain areas have different levels or types of parasite infestation, in order to (1) assess the risk that they are exposed to, which may influence the need for different conservation measures, and (2) understand whether the birds in these areas are adapted to these selective forces. Thus, we propose to return to Cape Verde in late October/early November, starting sampling on S. Nicolau or Fogo and ending sampling on Santiago in late December, focusing on sampling mountain birds (Monte Velha, Monte Tchota, Serra Malagueta).

6.5. Canto | Song

Para proceder à análise das diferenças entre os cantos dos machos das diferentes ilhas é necessário gravar mais cantos em S. Nicolau. Propomos regressar a esta ilha no final da época de reprodução, quando os machos ainda estão a defender activamente o território, e gravar cantos de pelo menos mais seis machos. Além disso, é necessário testar a reacção das aves de cada ilha aos cantos dos machos de outras ilhas com um método standardizado. Para tal, para cada ilha será criado um canto tipo, composto de partes de cantos de vários machos, e serão avaliadas as reacções dos machos a este canto tipo com um teste comportamental. Tudo isto deve ser feito no máximo no final da época de reprodução, caso contrário corre-se o risco de os machos já não estarem a defender o território e o teste não funcionar.

> To understand the differences between male songs from different islands it is necessary to record more songs on S. Nicolau. We propose to return to this island at the end of the breeding season, when males are still actively defending the territory, and record songs of at least six more males. Moreover, it is necessary to test the reaction of the birds from each island to the songs of males of other islands with a standardized method. To do this, we will create a "type" song for each island, composed of parts of several male songs from that island, and assess the reactions of other males to this type song with a behavioural test. All this must be done at the latest at the end of the breeding season, otherwise there is a risk that males are no longer defending the territory and the test does not work.

6.6. Dados de habitat | Habitat data

De modo a poder delinear medidas de conservação adequadas a esta espécie, é necessário recolher de forma sistematizada dados do habitat utilizados pela mesma. Assim, propomos fazer uma segunda época de campo em que este seja um dos objectivos principais. O protocolo deverá ser fácil e rápido de usar. Devem ser registados dados sobre factores bióticos e abióticos, bem como espécies de plantas presentes e tamanho do território, se possível. O tamanho do território só poderá ser estimado com confiança observando e registando movimentos de aves marcadas com anilhas coloridas. Caso contrário, será quase impossível distinguir as aves de territórios vizinhos. Neste sentido, recomendamos efectuar marcação de mais aves com anilhas coloridas, seguir os seus movimentos, marcando os pontos com GPS, e caracterizar o habitat das áreas utilizadas pelas mesmas. No entanto, aconselha-se que o seguimento das aves marcadas na época de campo passada seja já iniciado pelos técnicos locais, que assim conseguirão já os primeiros dados de uso do espaço por esta espécie. Estes dados são muito importantes para poder definir áreas com prioridade de conservação, e para construir modelos que possam ser usados para prever outras potenciais zonas de distribuição da espécie.

> To define successful conservation measures for this species, it is necessary to collect habitat data in a systematic way. We propose to do this as a main goal of a second field season. We should have a protocol easy and quick to use. We should record data on biotic and abiotic factors, plant species present and territory size, if possible. Territory size can only be estimated reliably observing and recording movements of colour-ringed birds. Otherwise, it will be almost impossible to distinguish birds from neighbouring territories. Hence, we recommend colour ringing more birds, following their movements, recording observation points with GPS and characterize the habitat of the areas they use. We also recommend that local technicians start recording observations of previously colour-ringed birds, and get the first data on spatial use of this species. These data are very important to

> define areas of conservation priority, and to build models that can be used to predict other potential areas of distribution of the species.

6.7. Métodos de censo | Censusing methods

Consideramos importante realizar censos de aves para obter uma estimativa mais correcta do número de indivíduos por ilha. Com base nas observações efectuadas em conjunto com vários técnicos cabo verdianos, recomendamos que sejam realizados pontos de escuta com duração de 10 minutos, em vez de transectos. Isto minimiza a probabilidade de não detectar aves que estejam presentes no território, já que estas parecem usar uma área grande, pelo menos após a época de reprodução, movimentando-se bastante e nem sempre emitindo sons. Sugerimos fazer este trabalho em conjunto com os técnicos locais de modo a melhorar e homogeneizar a metodologia. A altura mais indicada será na época de reprodução, uma vez que os machos deverão estar a vocalizar bastante para defender os territórios e estes deverão manter-se razoavelmente estáveis. Propomos que no caso específico de S. Nicolau, tratando-se de uma população reduzida e ainda pouco conhecida, sejam efectuados vários pontos de escuta dentro de cada território, com recurso a gravações, com uma duração mínima de 15 minutos, e de preferência repetidos várias vezes por ano, mesmo no caso de não haver resposta das aves nas primeiras vezes. Uma metodologia deste género foi utilizada pela equipa e pela técnica do Parque Natural de Monte Gordo, Maria Auxiliadora do Nascimento, em S. Nicolau e revelou-se a única forma de encontrar as aves mais elusivas.

> It is important to conduct bird censuses to obtain a more accurate estimate of the number of individuals per island. Based on the observations made we recommend the use of point counts lasting 10 minutes instead of transects. This minimizes the probability of not detecting birds that are present in the territory, as they seem to use a large area, at least after the breeding season, moving a lot and not always vocalizing. We recommend doing this work together with local technicians to improve and standardize the methodology. This should be done during breeding season, since males should be quite actively defending territories and these are expected to remain fairly stable. In the specific case of S. Nicolau, with a reduced and still understudied population, multiple point counts should be conducted within each territory, using playbacks, with a minimum duration of 15 minutes, and preferably repeated several times per year, even if there is no response of the birds on the first try. An approach of this kind was used by our team and the biologist Maria Auxiliadora do Nascimento, on the Natural Park of Monte Gordo on S. Nicolau, and proved the only way to find many cryptic birds.

Anexo | Appendix

Anexo I – Interpretação da informação constante na base de dados de campo da felosa de Cabo Verde Acrocephalus brevipennis

Antes de recolher dados de qualquer ave, é necessário identificá-la para se saber exactamente a que indivíduo pertencem os dados recolhidos. As anilhas de metal permitem identificar as aves com precisão atraves do uso de um número único para cada indivíduo, num suporte de metal duradouro e resistente; esta identificação é especialmente útil quando as aves são recapturadas. As anilhas de cor permitem identificar as aves em liberdade, sem necessitar de capturas, e seguir os movimentos das mesmas dentro dos seus territórios. O diâmetro interno das anilhas usadas para identificar a felosa de Cabo Verde *Acrocephalus brevipennis* é de 2,3 mm, adequado ao tamanho do tarso destes e de grande parte dos outros Passeriformes. Tanto as anilhas de metal como as de cor foram sempre colocadas no tarso das aves. As anilhas de metal utilizadas foram especialmente criadas para Cabo Verde, com a inscrição *M. Amb. C. Verde* (Ministério do Ambiente de Cabo Verde). O esquema de anilhagem está integrado na plataforma *European Colour-ring Birding*, que divulga e recolhe informação sobre observações de aves com anilhas coloridas. Esta plataforma pode ser acedida através do site http://www.cr-birding.org/.

Para cada ave capturada, a fórmula da asa foi avaliada por meio da identificação da pena primária mais longa e da emarginação observada nas penas primárias (dados não disponibilizados). A época de reprodução foi avaliada pelo registo da presença e estado de uma pelada de incubação. A muda das penas foi avaliada registando o estado de muda de cada ave de acordo com o sistema da British Trust for Ornithology (BTO) e registando o número de novas penas primárias e secundárias. A idade das aves (juvenil ou adulto) foi determinada pela observação do desgaste e estado de muda das penas e pela cor dos olhos, de acordo com o sistema da mesma organização. A cor dos olhos das aves foi registada com fotografias e categorizada entre cinzento, castanho ou vermelho. O sexo foi determinado pela observação do comportamento das aves, do seu tamanho, presença da pelada de incubação ou tamanho da protuberância cloacal (a ser comparado com os resultados da sexagem molecular). A presença de parasitas (ácaros) nas penas foi registada usando uma escala de A a G, e foram recolhidas fezes quando possível. Não foram registados quaisquer dados comportamentais, excepto a resposta dos machos capturados às gravações e se as aves manuseadas tentavam morder (dados não disponibilizados).

Captura

Número do registo de captura da ave.

Ilha

Ilha em que a ave foi capturada. ST = Santiago; FG = Fogo; SN = S. Nicolau.

Anilhas de cor

Código de anilhas de cor usado para identificar a ave. Os códigos de anilhas de cor lêem-se da direita para a esquerda e de cima para baixo. Assim, numa ave com o código RX_BY, RX refere-se à pata direita da ave e BY à pata esquerda da ave, ambas lidas de cima para baixo. Esta ave terá as seguintes anilhas: uma anilha vermelha (R) no topo do tarso direito, uma anilha de metal (X) na parte de baixo do tarso direito, uma anilha azul (B) no topo do tarso esquerdo e uma anilha amarela (Y) na parte de baixo do tarso esquerdo (Figura 9).

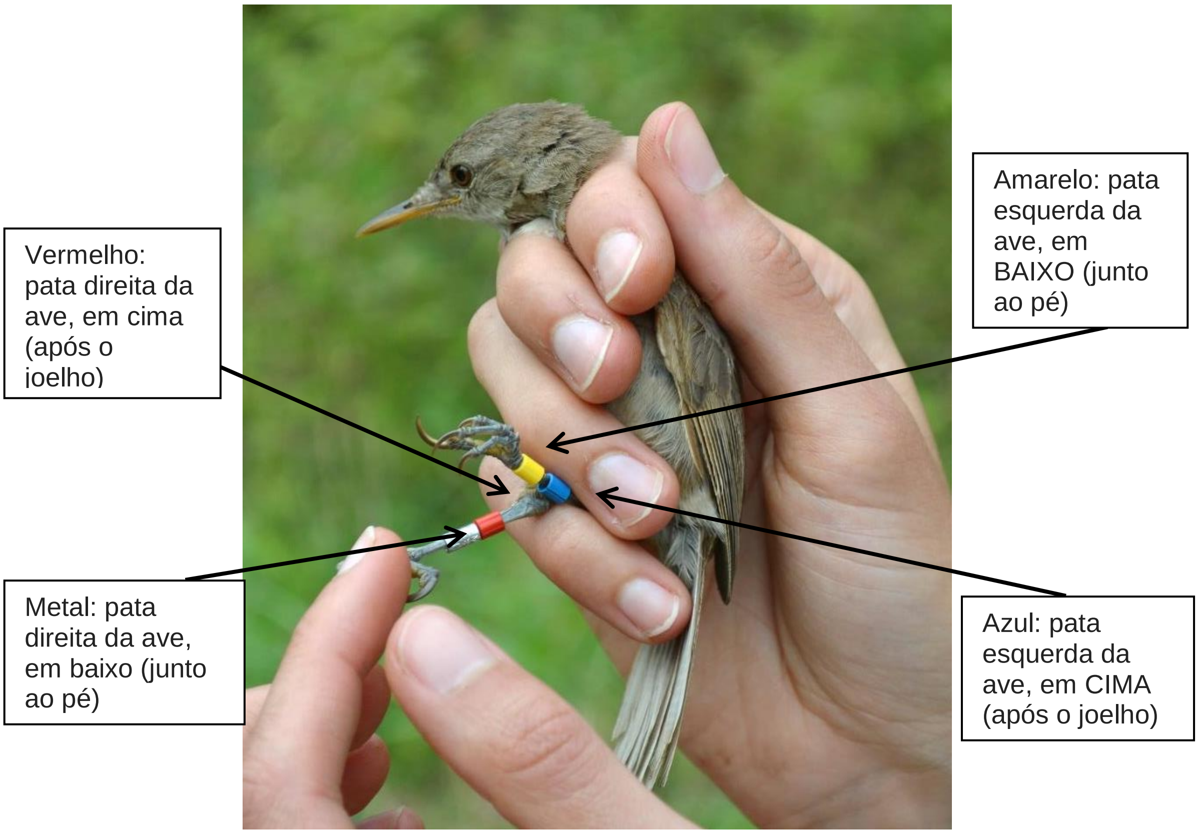


Figure 9 – Diagrama ilustrando as posições das anilhas coloridas e da anilha de metal nas patas de uma felosa de Cabo Verde *Acrocephalus brevipennis*: exemplo de ave anilhada em Santiago.

Repare-se que, na Figura 9, a ave está posicionada de um modo em que parece que a anilha amarela é a anilha de cima da pata equerda da ave. Na realidade, e observando com atenção, a anilha amarela esta junto ao pé da ave. Logo, se a ave estiver na sua posição natural poderá confirmar-se que a anilha amarela está em baixo, junto ao pé, e não em cima, após o joelho, como poderá parecer à primeira vista. Estes pormenores são muito importantes para a identificação correcta do código de cores usado pela ave, já que um leitura errada deste código implica uma identificação incorrecta e consequentemente registos de campo errados. Por exemplo, a ave RX_BY referida no exemplo, capturada junto ao tanque de S. Jorge no dia 27 de Novembro de 2013, poderá ser confundida com a ave RX_YB, capturada tambem em S. Jorge mas mais abaixo, no vale com a plantação de mandioca, no dia 3 de Dezembro de 2013. É, por isso, essencial ter um cuidado extremo no registo do código de cores observado, e evitar registar códigos de cores em que possam haver dúvidas, ou pelo menos fazer sempre uma nota sobre isso. Segundo as convenções usadas geralmente na Europa, os códigos estão escritos com as iniciais de cada cor em inglês (ver tabela VI).

Table VI – Cores usadas na identificação das felosas de Cabo Verde *Acrocephalus brevipennis* na época de campo 2013/2014, abreviaturas usadas no código, e possíveis posições na pata das aves.

Cor	Abreviatura	Pata
Dark blue 🟦	**B**	esquerda ou direita
White ⬜	**W**	esquerda ou direita
Orange 🟧	**O**	esquerda ou direita
Red 🟥	**R**	esquerda ou direita
Yellow 🟨	**Y**	esquerda ou direita
Anilha de metal Cabo Verde (Ministério do Ambiente)	**X**	Santiago: direita em baixo Fogo: esquerda em baixo S. Nicolau: esquerda em cima

Anilha de metal

Número completo da anilha de metal usada para identificar a ave.

Zona

Zona da ilha onde a ave foi capturada; refere-se apenas às localizações dentro da ilha de Santiago.

Local

Código do ponto de GPS do local onde a ave foi capturada.

Data

Data da captura.

Hora

Hora a que a ave caiu na rede.

Idade

Idade estimada da ave, em relação ao ano civil em que a mesma foi capturada, segundo o sistema EURING (tabela VII). Os valores desta escala não indicam os anos de vida da ave mas o grau de certeza em relação ao ano de nascimento da ave. Por exemplo, se temos a certeza que uma ave nasceu no corrente ano civil, o código da idade será 3 (i.e., juvenis de Santiago e Fogo nascidos e capturados em 2013). Se temos a certeza que não nasceu neste ano, mas não sabemos ao certo em que ano foi, a idade será 4 (i.e., adultos de Santiago e Fogo capturados em 2013). Se temos a certeza que a ave nasceu no ano civil passado, o código sera 5 (i.e., juvenis de S. Nicolau, nascidos na época de reprodução de 2013 e capturados em 2014). Se sabemos com certeza que não nasceu neste ano, nem no ano passado, mas não sabemos dizer há quantos anos nasceu, o código será 6 (i.e., adultos de S. Nicolau capturados em 2014). Se a ave já está no estado de desenvolvimento de adulto mas é impossível determinar com precisão em que ano nasceu, o código será 2. Na prática, os valores 3 e 5 indicam que a ave é juvenil, os valores 4 e 6 indicam que a ave é adulta, e o valor 2 indica que a idade é deconhecida. Para avaliar a idade das aves há que ter em conta vários critérios, como a cor da íris, o estado da muda, a presença de plumagem juvenil, o desenvolvimento do bico, a presença de pontos na língua, a presença de pelada de incubação, a cor das penas, entre outros critérios.

Table VII – Códigos usados para registar a idade das aves em relação ao ano de captura. Tabela adaptada de Bairlein (1994).

0	Idade desconhecida, i.e., não registada.
1	Pullus: cria ainda no ninho, incapaz de voar independenmente e que se consegue apanhar com a mão.
2	Ave totalmente desenvolvida e capaz de voar, mas de idade desconhecida.
3	Primeiro ano: ave totalmente desenvolvida nascida na época de reprodução do corrente ano civil.
4	Após o primeiro ano: ave totalmente desenvolvida nascida antes da época de reprodução do corrente ano civil; ano de nascimento desconhecido.
5	Segundo ano: ave nascida no ano civil anterior ao corrente.
6	Após o segundo ano: ave totalmente desenvolvida nascida antes do ano civil anterior ao corrente; ano de nascimento desconhecido.
7	Terceiro ano: ave nascida dois anos antes do ano civil corrente.
8	Após o terceiro ano: ave totalmente desenvolvida nascida dois ou mais anos antes do ano corrente; ano de nascimento desconhecido.
9	Quarto ano: ave nascida três anos antes do ano civil corrente.
A	Após o quarto ano de vida, mas ano exacto de nascimento desconhecido.

Cor da íris

Cor da íris dos olhos das aves como avaliada pelo anilhador. Esta avaliação poderá ser subjectiva, mas fornece mais um critério para avaliar a idade das aves. Nas aves do género *Acrocephalus* e outras felosas, verifica-se frequentemente que os juvenis tem os olhos mais acinzentados/esverdeados, e que esta cor muda gradualmente para castanho e avermelhado à medida que as aves se desenvolvem (Jenni & Winkler 2011; Kennerley & Pearson 2010). Apesar de não ser um indicador preciso da idade das aves, pode ser usado como critério complementar para estimar a idade das mesmas. Assim, provavelmente uma ave com olhos esverdeados será juvenil, com olhos castanhos estará já num estado de desenvolvimento de adulto e com olhos avermelhados possivelmente terá mais de um ano de idade (Figura 10).

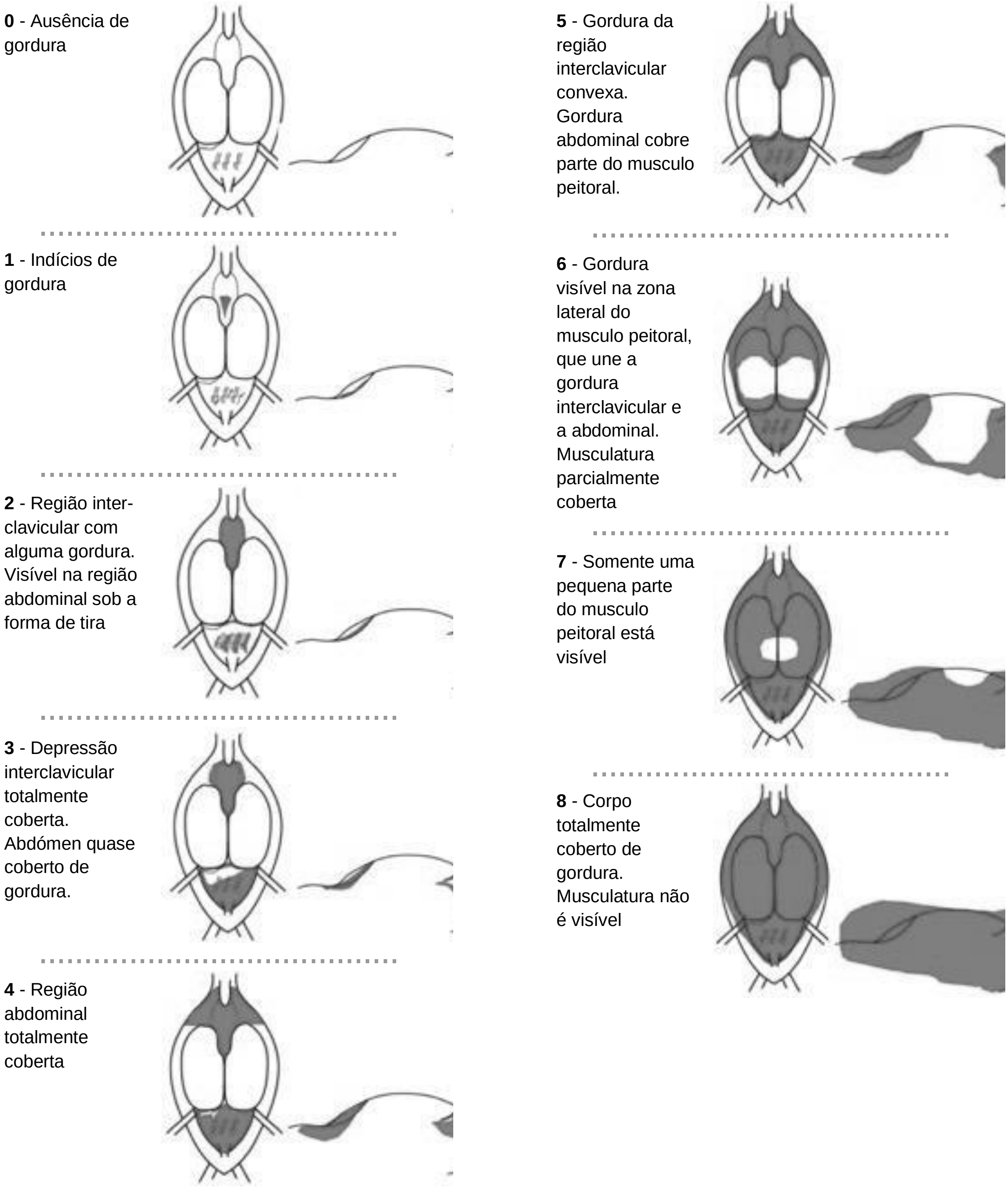
Figure 10 - Alteração da cor da íris das aves conforme a idade: os juvenis tem a íris acinzentada (esquerda), mudando para castanha (centro) e avermelhada (direita) à medida que se tornam adultos.

Sexo

Sexo da ave determinado por métodos de sexagem molecular – amplificação de um fragmento nos cromossomas sexuais ZW com o uso dos primers P2 e P8, segundo Griffiths et al. (1998).

Gordura

Acumulação de gordura no abdómen da ave (Figura 11), segundo a escala mais usada na Europa (Kaiser 1993). A acumulação de gordura indica principalmente se a ave está a acumular reservas para migração (Gauthreaux 1981), mas também é um bom indicador da condição física da ave.



0 - Ausência de gordura

1 - Indícios de gordura

2 - Região inter-clavicular com alguma gordura. Visível na região abdominal sob a forma de tira

3 - Depressão interclavicular totalmente coberta. Abdómen quase coberto de gordura.

4 - Região abdominal totalmente coberta

5 - Gordura da região interclavicular convexa. Gordura abdominal cobre parte do musculo peitoral.

6 - Gordura visível na zona lateral do musculo peitoral, que une a gordura interclavicular e a abdominal. Musculatura parcialmente coberta

7 - Somente uma pequena parte do musculo peitoral está visível

8 - Corpo totalmente coberto de gordura. Musculatura não é visível

Figure 11 – Escala usada para indicar a acumulação de gordura no abdómen dos Passeriformes. Adaptado de Kaiser (1993).

Músculo

Estado de desenvolvimento do músculo peitoral da ave, segundo a escala mais usada na Europa (tabela VIII, Figura 12). O desenvolvimento dos músculos da ave é um bom indicador do estado de saúde geral da mesma (quanto menos desenvolvido, mais magra, mal nutrida e pouco resistente estará a ave). Para avaliar o desenvolvimento do músculo, o investigador deve soprar fortemente no abdómen da ave, para que as penas se afastem naturalmente (Figura 13).

Table VIII – Descrição da condição dos músculos peitorais da ave para cada valor da escala de avaliação segundo Bairlein (1994).

0	Esterno sobressaído; músculos deprimidos.
1	Esterno fácil de distinguir mas não sobressaído; músculos nem deprimidos nem desenvolvidos.
2	Esterno ainda discernível; músculos ligeiramente desenvolvidos (arredondados).
3	Esterno praticamente impossível de distinguir devido a músculos bem desenvolvidos e arredondados.

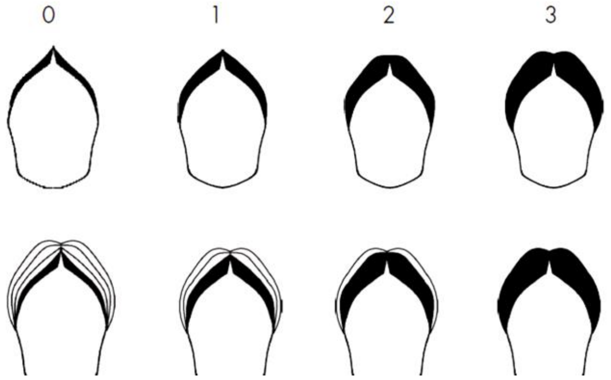
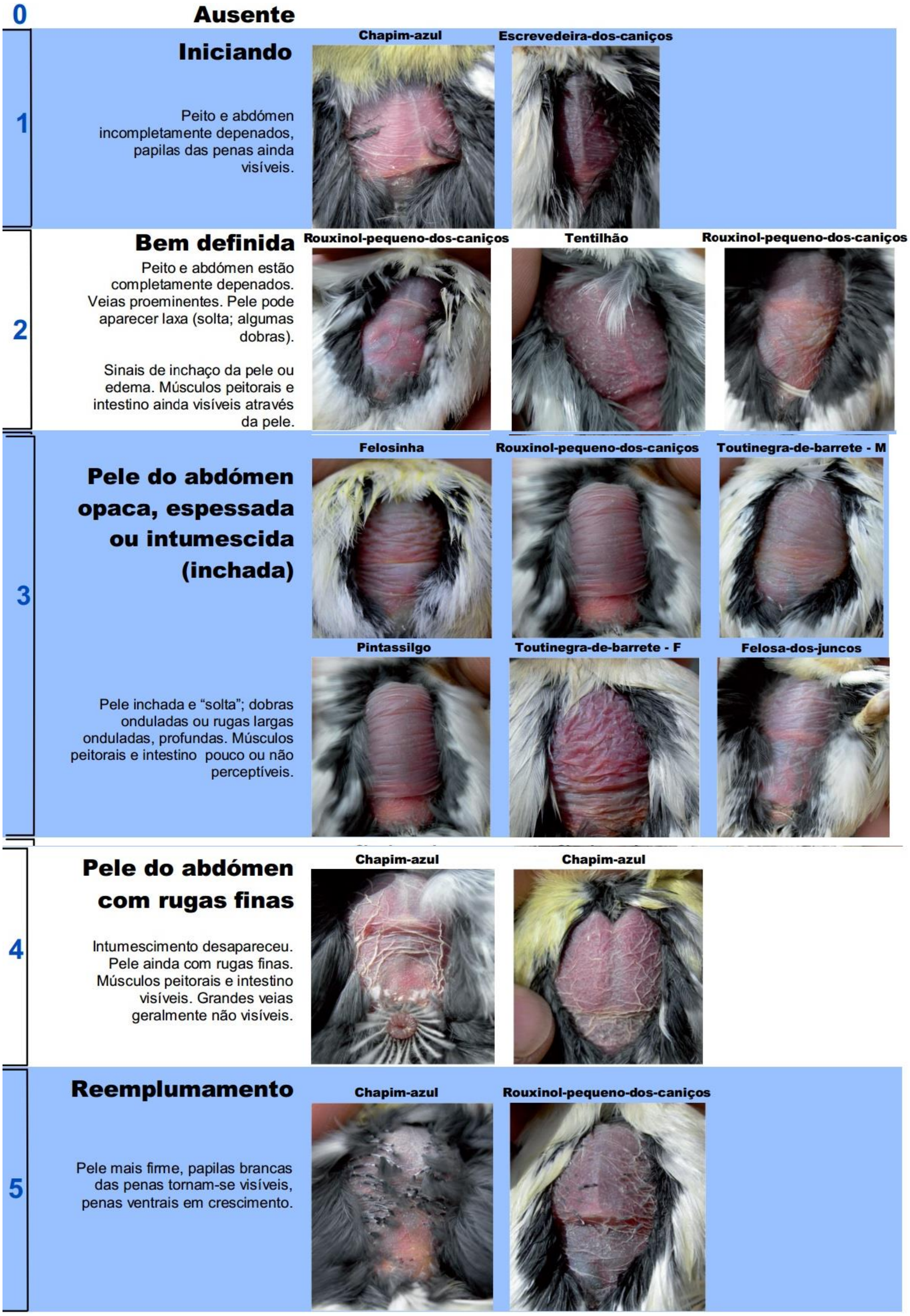


Figure 12 – Esquema do desenvolvimento do músculo peitoral nos Passeriformes com respectiva escala de classificação (acima) e ilustração das diferenças entre as classificações (abaixo). Adaptado de Barlein (1994).

Figure 13 – Investigador soprando no abdómen de uma felosa de Cabo Verde *Acrocephalus brevipennis* para avaliar o desenvolvimento do músculo peitoral. Foto de Jaelsa Moreira.

Pelada de incubação

A pelada de incubação é uma área de pele sem penas no abdómen de uma ave que está a incubar os ovos, apresentando além disso um aumento dos vasos sanguíneos locais para facilitar a transferência de calor do corpo do progenitor para os ovos. Após a incubação, a pelada regride e o abdómen da ave volta a ficar coberto por penas. O estado de desenvolvimento ou regressão da pelada é um indicador bastante fiável da fase da época de reprodução em que a ave se encontra. Isto é, se a ave está a perder as penas, está a iniciar a fase de deposição dos ovos; se tem uma pelada bem evidente, está a incubar; se a pelada está a ficar recoberta por penas, a incubação dos ovos terminou ou está a terminar. Para observar a pelada de de incubação, o invetigador sopra nas penas do abdómen da ave, tal como para avaliar o músculo (Figura 13). Para este trabalho utilizámos uma escala de 0 a 5 (Figura 14). É necessário ter cuidado para não confundir uma verdadeira pelada de incubação com a ausência de penas na zona ventral observada em aves juvenis, que ainda não adquiriram a plumagem completa, ou em adultos em muda (Figura 15).



Peito e abdómen incompletamente depenados, papilas das penas ainda visíveis.

Peito e abdómen estão completamente depenados. Veias proeminentes. Pele pode aparecer laxa (solta; algumas dobras).

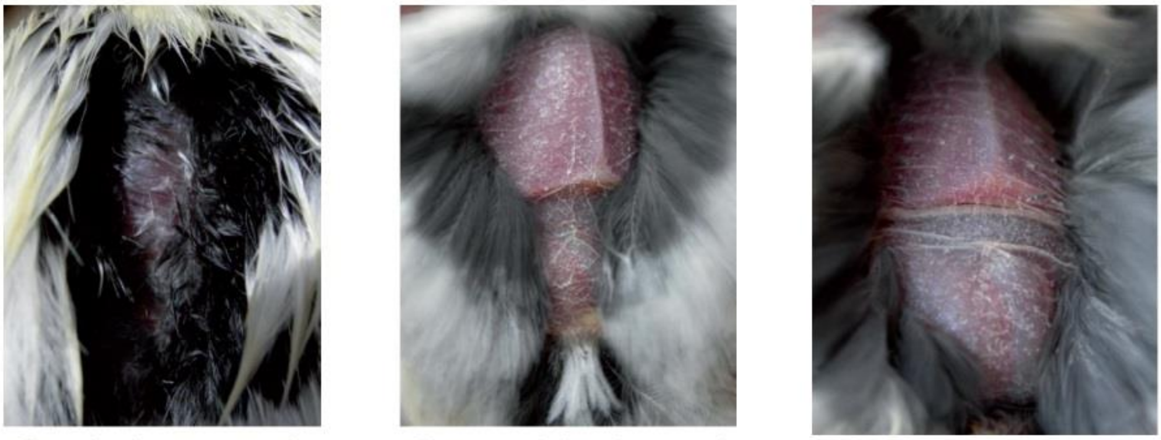
Sinais de inchaço da pele ou edema. Músculos peitorais e intestino ainda visíveis através da pele.

Pele inchada e "solta"; dobras onduladas ou rugas largas onduladas, profundas. Músculos peitorais e intestino pouco ou não perceptíveis.

Intumescimento desapareceu. Pele ainda com rugas finas. Músculos peitorais e intestino visíveis. Grandes veias geralmente não visíveis.

Pele mais firme, papilas brancas das penas tornam-se visíveis, penas ventrais em crescimento.

Figure 14 - Estádios de desenvolvimento e regressão da pelada de incubação em Passeriformes. Retirado do site da Associação Portuguesa de Anilhadores de Aves (APAA).



Rouxinol-pequeno-dos-caniços - macho, não se trata de uma pelada de incubação.
Penas a cobrir a pele.
Observe o enrugamento da pele.

Escrevedeira-dos-caniços - 3J, não se trata de uma pelada de incubação.
Observe algum enrugamento sobre o intestino.

Toutinegra-de-barrete - 3J, início da muda pós-juvenil.

Chapim-real - macho, em muda geral.

O mesmo chapim-real - macho, em muda geral, com penas ventrais afastadas: reemplumamento do peito e abdómen, mas não corresponde a uma pelada de incubação.

Carriça - 3j.

Figure 15 – Exemplos de identificações erradas de pelada de incubação. Retirado do site da Associação Portuguesa de Anilhadores de Aves (APAA).

Asa

Comprimento da asa em milímetros, medido desde a dobra da asa até a ponta da rémige primária mais longa (Figura 16). Para este projecto, o comprimento é medido usando o método “maximum wing chord” descrito por Svensson (1992). Segundo este método, a dobra da asa é encostada à extremidade zero da régua, as penas achatadas contra a régua e endireitadas ao longo da mesma. São feitas duas medições para detectar e corrigir eventuais erros.

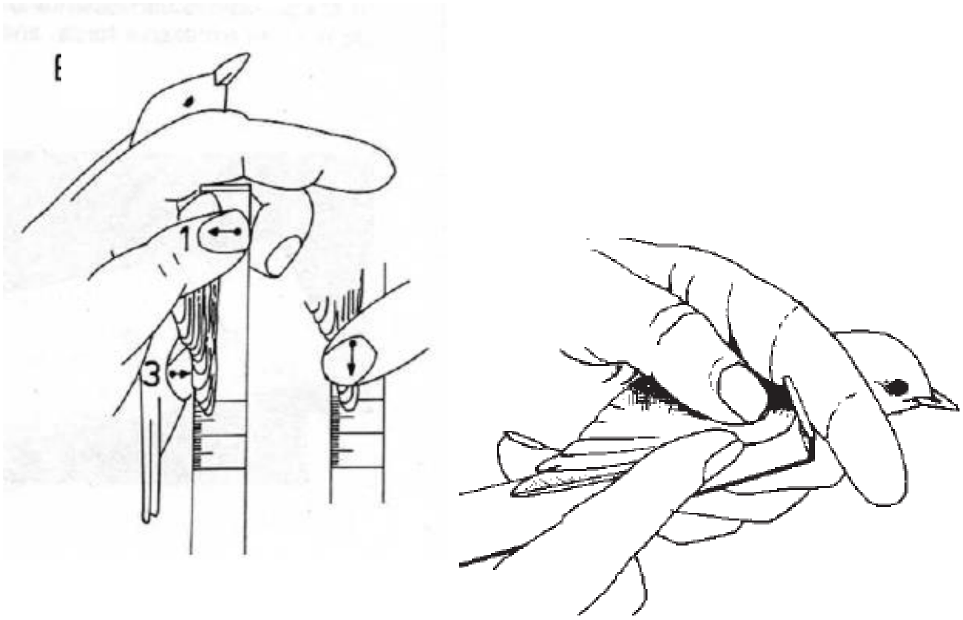


Figure 16 – Medição do comprimento da asa com o método “maximum wing chord”; imagem retirada de Redfern & Clark (2001).

Tarso direito

Comprimento do osso metatarso direito, medido desde a extremidade proximal até à distal, na zona da dobra com o pé. Para este projecto, mede-se apenas o comprimento do osso, aproximado à décima de milímetro, segundo o método descrito por Svensson (1992). O paquímetro encosta na extremidade do osso, com a pata dobrada, e na outra extremidade com o pé também dobrado, sem encostar na tíbia ou no pé da ave (Figura 17). Esta medição também é feita duas vezes.

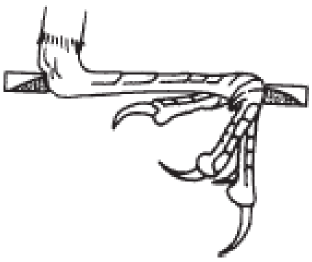


Figure 17 - Medição do comprimento do tarso com o método “minimum tarsus length”; imagem retirada de Redfern & Clark (2001).

Cabeça e bico

Comprimento total da cabeça e bico, medidos desde a extremidade posterior da cabeça até à ponta do bico, em linha recta (Figura 18). Esta medida também é tirada com o paquímetro, aproximando à décima de milímetro. Esta medição também é feita duas vezes.

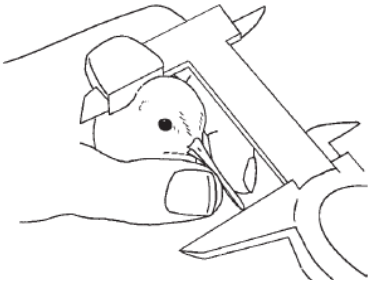


Figure 18 - Medição do comprimento total da cabeça e bico; imagem retirada de Redfern & Clark (2001).

Bico

Comprimento do bico, em milímetros, desde a ponta até à extremidade anterior da narina (Figura 19). Medido com o paquímetro até à décima de milímetro. Esta medição também é feita duas vezes.

Figure 19 – Investigador medindo o comprimento do bico (até à narina) de uma felosa de Cabo Verde *Acrocephalus brevipennis*. Foto de Jaelsa Moreira.

Profundidade do bico

Profundidade do bico, em milímetros, encostando o paquímetro no local onde as penas terminam na mandíbula superior e medindo num ângulo de 90° em relação ao plano horizontal do bico. Medido com o paquímetro até à décima de milímetro. Esta medição também é feita duas vezes.

Largura do bico

Largura máxima do bico, em milímetros, medida no mesmo ponto de referência que a profundidade (neste caso, onde terminam as penas; Figura 20). Medido com o paquímetro até à décima de milímetro. Esta medição também é feita duas vezes.

Figure 20 – Investigador medindo a largura do bico de uma felosa de Cabo Verde *Acrocephalus brevipennis*. Foto de Jaelsa Moreira.

Muda

o estado de progressão da muda da ave capturada. Para este projecto, a muda foi registada segundo a escala mais usada na Europa. Esta escala é diferente para aves no seu primeiro ano de vida (juvenis) ou para adultos (ver tabela IX).

Table IX – Códigos usados para classificar o estádio da muda das penas das aves, em aves juvenis (coluna da esquerda) e adultas (direita).

Juveniles		**Adults**	
J	Ave completamente em plumagem juvenil. Aplica-se a todos os Passeriformes juvenis antes da muda pós-juvenil.	**O**	Plumagem velha, sem muda nas penas do corpo ou de vôo.
P	Muda pós-juvenil das penas do corpo.	**B**	Adulto com muda das penas corporais, mas sem estar na fase de muda principal das penas de vôo (asas e cauda).
O	Juvenis que tenham completado uma muda parcial das penas do corpo, mas ainda possuam penas de vôo (coberturas, asa ou cauda) velhas.	**M**	Muda principal: penas das asas ou cauda em muda activa.
M	Juvenis com muda activa de penas de vôo.	**S**	A iniciar a muda das penas do corpo – penas das asas e cauda velhas.
A	Juvenis com muda das penas das asas (primárias ou secundárias) interrompida.	**E**	A terminar a muda das penas do corpo – penas das asas e cauda novas.
T	Mudando apenas as penas terciárias ou da cauda, em muda pós-juvenil parcial.	**N**	Plumagem nova após terminar a muda.

Primárias novas

Rémiges primárias novas, numeradas ascendentemente (começando da pena mais exterior e progredindo em direcção ao corpo da ave). Note-se que na maioria das espécies, incluindo a felosa de Cabo Verde, as primárias começam a mudar descendentemente, ou seja, da mais interior progredindo até ao extremo exterior da asa. Assim, uma ave com as penas 8 a 10 novas (Figura 21 esquerda) estará num estado de muda menos avançado que uma ave com as penas 3 a 10 novas (Figura 21 direita).

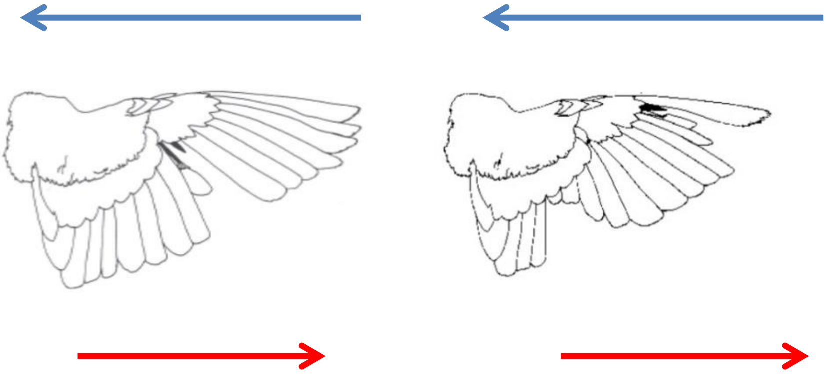


Figure 21 – Asa de ave com as rémiges primárias 10 e 9 novas, e as restantes velhas (esquerda); asa de ave com as rémiges primárias 10 a 3 novas, e as restantes velhas (direita); a alula (pena número 1, mais exterior) não esta visível nestas imagens; as setas azuis indicam a direcção da numeração das penas, e as setas vermelhas indicam a direcção da progressão da muda; imagem adaptada de Redfern & Clark (2001).

Secundárias novas

Rémiges secundárias novas, numeradas ascendentemente (começando da pena mais exterior e progredindo em direcção ao corpo da ave). Note-se que, ao contrário das penas primárias, na felosa de Cabo Verde, as secundárias começam a mudar ascendentemente, ou seja, da mais exterior progredindo até as terciárias, junto do corpo. Assim, uma ave com as penas 1 a 4 novas estará num estado de muda mais avançado que uma ave com as penas 1 a 2 novas. Na felosa de Cabo Verde, as secundárias comecam a mudar depois das primárias já estarem a mudar (Figura 22).

Figure 22 – Asa de felosa de Cabo Verde *Acrocephalus brevipennis* a mudar as penas primárias, secundárias e coberturas. Observe-se a diferença de cor entre as penas velhas (mais desgastadas e claras) e novas (mais frescas e escuras). Esta ave tem as penas primárias 1 a 5 velhas, 6 a 10 novas ou em crescimento, a secundária 1 a crescer e as restantes velhas.

Parasitas das penas

Quantidade de ectoparasitas das penas das asas, estimada numa escala de A a G (tabela X, Figura 23).

Table X – Escala para os ectoparasitas das penas das asas.

Número de ectoparasitas	Classificação
0	A
1	B
2-5	C
6-10	D
11-20	E
21-100	F
100+	G

Figure 23 – Exemplo de asa mostrando infestação de parasitas das penas observada na maior parte das felosas de Cabo Verde *Acrocephalus brevipennis* amostradas: valor F na escala.

Peso

Peso da ave, em gramas. Medido numa balança até à décima da grama.

Anexo II – Dados biométricos e outros recolhidos em cada felosa de Cabo Verde Acrocephalus brevipennis capturada

Table XI – Dados de felosas de Cabo Verde *Acrocephalus brevipennis* capturadas: anilhas, local (com coordenadas prontas a inserir no Google Earth), data e hora, idade, sexo, gordura, músculo e pelada de incubação; na = dados não registados.

Captura #	Anilhas de cor	Anilha de metal	Ilha	Zona	Local	Coordenadas Google Earth	Data	Hora	Idade	Cor da íris	Sexo	Gordura	Músculo	Pelada de incubação
1	RX_RR	A000002	ST	Centro	AB017	15.07297°,-23.55918°	24/11/2013	17:50	3	na	macho	1	2	ausente
2	RX_RB	A000005	ST	Centro	AB012	15.0509°,-23.60833°	25/11/2013	08:45	4	vermelho	macho	0	3	ausente
3	RX_RW	A000011	ST	Centro	AB013	15.05092°,-23.60945°	25/11/2013	14:00	4	vermelho	macho	0	3	ausente
4	RX_RY	A000012	ST	Centro	AB013	15.05092°,-23.60945°	25/11/2013	14:40	3	cinzento	fêmea	2	3	ausente
5	RX_BR	A000016	ST	Centro	AB012	15.0509°,-23.60833°	26/11/2013	09:52	4	vermelho	macho	0	3	ausente
6	RX_RO	A000017	ST	Centro	AB005B	15.05065°,-23.60403°	26/11/2013	13:25	4	vermelho	macho	1	3	ausente
7	RX_BB	A000018	ST	Centro	AB005B	15.05065°,-23.60403°	26/11/2013	14:10	4	vermelho	fêmea	0	2	na
8	RX_BW	A000019	ST	Centro	AB009	15.0472°,-23.60558°	26/11/2013	17:00	4	vermelho	fêmea	na	na	na
9	RX_BY	A000023	ST	Centro	AB012	15.0509°,-23.60833°	27/11/2013	08:40	4	vermelho	fêmea	0	3	na
10	RX_BO	A000025	ST	Centro	AB022	15.04264°,-23.60569°	27/11/2013	13:15	4	vermelho	macho	1	3	ausente
11	RX_WR	A000027	ST	Centro	AB009	15.0472°,-23.60558°	27/11/2013	17:50	4	na	macho	2	3	ausente
12	RX_WB	A000028	ST	Norte	AB031	15.22911°,-23.73972°	29/11/2013	12:30	4	vermelho	macho	2	2	ausente
13	RX_WW	A000029	ST	Centro	AB033	15.13217°,-23.52942°	30/11/2013	12:20	4	vermelho	macho	0	2	ausente
14	RX_WY	A000030	ST	Sul	AB035	14.92108°,-23.60074°	02/12/2013	10:15	3	castanho	fêmea	0	2	ausente
15	RX_WO	A000031	ST	Sul	AB036	14.92407°,-23.60145°	02/12/2013	13:30	3	castanho	macho	0	2	ausente
16	RX_YR	A000032	ST	Sul	AB036	14.92407°,-23.60145°	02/12/2013	13:30	3	castanho	fêmea	0	2	ausente
17	RX_YB	A000034	ST	Centro	AB010B	15.05243°,-23.60762°	03/12/2013	11:45	3	verde	fêmea	0	2	ausente
18	RX_YW	A000035	ST	Centro	AB010B	15.05243°,-23.60762°	03/12/2013	11:45	4	vermelho	fêmea	1	2	4
19	RX_YY	A000036	ST	Centro	AB010B	15.05243°,-23.60762°	03/12/2013	12:30	4	vermelho	macho	0	3	ausente
20	RX_YO	A000038	ST	Centro	AB010	15.05224°,-23.60744°	03/12/2013	15:04	4	castanho	macho	1	3	ausente
21	RX_OR	A000039	ST	Centro	AB010	15.05224°,-23.60744°	03/12/2013	15:04	4	photo	fêmea	na	na	3
22	RX_OB	A000042	ST	Centro	AB010	15.05224°,-23.60744°	03/12/2013	16:20	4	castanho	fêmea	1	3	2

Captura #	Anilhas de cor	Anilha de metal	Ilha	Zona	Local	Coordenadas Google Earth	Data	Hora	Idade	Cor da íris	Sexo	Gordura	Músculo	Pelada de incubação
23	RX_OW	A000043	ST	Centro	AB010	15.05224°,-23.60744°	03/12/2013	16:35	2	castanho	macho	2	3	ausente
24	RX_OY	A000045	ST	Sul	AB003	14.92825°,-23.59861°	04/12/2013	11:35	2	castanho	macho	0	2	ausente
25	RX_OO	A000046	ST	Sul	AB039	14.93402°,-23.5991°	04/12/2013	15:05	3	cinzento/verde	fêmea	2	2	ausente
26	BX_RR	A000047	ST	Sul	AB039	14.93402°,-23.5991°	04/12/2013	16:10	4	castanho	fêmea	0	2	ausente
27	BX_RB	A000048	ST	Sul	AB039	14.93402°,-23.5991°	04/12/2013	16:10	4	castanho	macho	0	2	ausente
28	BX_RW	A000050	ST	Centro	AB001	15.050722°,-23.603543°	05/12/2013	15:34	4	castanho	macho	1	3	ausente
29	BX_RY	A000051	ST	Centro	AB001	15.050722°,-23.603543°	05/12/2013	15:34	4	castanho	fêmea	1	2	ausente
30	BX_RO	A000054	ST	Centro	AB040	15.05299°,-23.60622°	07/12/2013	17:00	4	vermelho	macho	0	3	ausente
31	BX_BR	A000055	ST	Centro	AB040	15.05299°,-23.60622°	07/12/2013	17:25	4	vermelho	fêmea	1	2	5
32	BX_BB	A000056	ST	Centro	AB041	15.07367°,-23.55428°	08/12/2013	17:22	4	castanho	macho	0	2	5
33	BX_BW	A000057	ST	Centro	AB041	15.07367°,-23.55428°	08/12/2013	17:22	3	verde	fêmea	0	2	ausente
34	BX_BY	A000058	ST	Centro	AB041	15.07367°,-23.55428°	08/12/2013	18:15	3	verde	macho	0	2	ausente
35	BX_BO	A000059	ST	Centro	AB041	15.07367°,-23.55428°	08/12/2013	18:30	4	castanho	fêmea	0	2	5
36	BX_WR	A000061	ST	Norte	AB042	15.20632°,-23.65713°	09/12/2013	12:11	4	castanho	macho	1	3	ausente
37	BX_WB	A000062	ST	Norte	AB043	15.24336°,-23.65592°	09/12/2013	17:25	4	castanho	macho	0	3	ausente
38	BX_WW	A000063	ST	Norte	AB044	15.23974°,-23.65415°	10/12/2013	12:30	4	vermelho	macho	0	2	ausente
39	BX_WY	A000065	ST	Norte	AB044	15.23974°,-23.65415°	10/12/2013	13:10	4	vermelho	fêmea	0	3	4
40	BX_WO	A000066	ST	Norte	AB045	15.20888°,-23.66544°	10/12/2013	16:10	4	castanho	macho	1	3	ausente
41	BX_YR	A000067	ST	Norte	AB046	15.19793°,-23.67876°	11/12/2013	09:10	4	castanho	macho	0	3	ausente
42	BX_YB	A000068	ST	Norte	AB046	15.19793°,-23.67876°	11/12/2013	09:25	4	castanho	fêmea	0	2	4
43	BX_YW	A000070	ST	Norte	AB047	15.18919°,-23.68171°	11/12/2013	11:45	4	vermelho	macho	0	3	ausente
44	BX_YY	A000073	ST	Norte	AB049	15.18016°,-23.67312°	12/12/2013	13:22	4	castanho	macho	0	3	ausente
45	BX_YO	A000074	ST	Norte	AB029B	15.17854°,-23.6732°	12/12/2013	14:50	4	castanho	macho	0	3	ausente
46	BX_OR	A000076	ST	Centro	AB050	15.03443°,-23.61415°	14/12/2013	17:44	4	castanho	macho	1	3	ausente
47	BX_OB	A000077	ST	Norte	AB029	15.17875°,-23.67276°	15/12/2013	15:30	4	castanho	macho	0	3	ausente
48	BX_OW	A000080	ST	Norte	AB052	15.18078°,-23.67272°	16/12/2013	11:25	4	castanho	fêmea	na	na	3
49	BX_OY	A000081	ST	Norte	AB053	15.18156°,-23.67242°	16/12/2013	12:42	4	vermelho	macho	0	3	ausente

Captura #	Anilhas de cor	Anilha de metal	Ilha	Zona	Local	Coordenadas Google Earth	Data	Hora	Idade	Cor da íris	Sexo	Gordura	Músculo	Pelada de incubação
50	BX_OO	A000082	ST	Norte	AB053	15.18156°,-23.67242°	16/12/2013	13:08	4	castanho	fêmea	0	2	4
51	RR_RX	A000085	FG	na	AB054	15.02021°,-24.32742°	19/12/2013	15:50	4	castanho	macho	1	3	ausente
52	RR_BX	A000086	FG	na	AB055	15.01898°,-24.32777°	20/12/2013	12:54	4	castanho	macho	0	2	ausente
53	RR_WX	A000087	FG	na	AB056	15.01835°,-24.32909°	21/12/2013	09:35	4	castanho	macho	0	2	ausente
54	RR_YX	A000088	FG	na	AB058	15.01718°,-24.32908°	21/12/2013	13:45	4	castanho	macho	0	3	ausente
55	RR_OX	A000089	FG	na	AB060	15.01866°,-24.33018°	22/12/2013	12:00	4	vermelho	macho	1	2	ausente
56	RB_RX	A000090	FG	na	AB061	15.01795°,-24.3306°	22/12/2013	14:45	4	castanho	macho	0	3	ausente
57	RB_BX	A000091	FG	na	AB062	15.01762°,-24.32099°	22/12/2013	16:00	4	castanho	macho	2	2	5
58	RB_WX	A000093	FG	na	AB063	15.01753°,-24.3309°	22/12/2013	17:25	4	castanho	fêmea	2	2	5
59	RB_YX	A000094	FG	na	AB064	15.01967°,-24.32881°	23/12/2013	09:00	4	castanho	fêmea	0	2	5
60	RB_OX	A000095	FG	na	AB064	15.01967°,-24.32881°	23/12/2013	09:40	4	castanho	macho	0	2	5
61	RW_RX	A000096	FG	na	AB065	15.01687°,-24.33145°	23/12/2013	12:05	2	castanho	macho	0	3	na
62	RW_BX	A000097	FG	na	AB065	15.01687°,-24.33145°	23/12/2013	13:20	4	castanho	fêmea	0	3	4
63	RW_WX	A000098	FG	na	AB066	15.0167°,-24.33208°	23/12/2013	14:49	2	castanho	fêmea	0	2	ausente
64	RW_YX	A000099	FG	na	AB067	15.0165°,-24.33195°	23/12/2013	16:40	4	castanho	macho	0	2	5
65	RW_OX	A000100	FG	na	AB073	15.023123°,-24.330723°	26/12/2013	12:05	4	castanho	macho	0	2	5
66	RY_RX	A000101	FG	na	AB074	15.02299°,-24.32901°	27/12/2013	09:27	2	castanho	macho	1	2	ausente
67	RY_BX	A000102	FG	na	AB074	15.02299°,-24.32901°	27/12/2013	09:55	3	verde/castanho	fêmea	0	2	ausente
68	removidas	A000103	FG	na	AB075	15.01989°,-24.32419°	27/12/2013	11:15	2	castanho	macho	2	3	5
69	RY_WX	A000104	FG	na	AB076	15.01998°,-24.32549°	27/12/2013	13:10	4	vermelho	macho	1	3	5
70	RY_YX	A000105	FG	na	AB077	15.01931°,-24.32527°	27/12/2013	15:15	3	verde/castanho	fêmea	0	2	ausente
71	RY_OX	A000106	FG	na	AB077	15.01931°,-24.32527°	27/12/2013	15:15	2	castanho	macho	0	2	ausente
72	RO_RX	A000107	FG	na	AB078	15.02381°,-24.32953°	28/12/2013	14:22	4	vermelho	macho	0	2	5
73	RO_BX	A000109	FG	na	AB078	15.02381°,-24.32953°	28/12/2013	15:20	2	castanho	macho	1	3	ausente
74	RO_WX	A000110	FG	na	AB080	15.01964°,-24.32666°	29/12/2013	10:00	4	castanho	macho	0	2	ausente
75	RO_YX	A000111	FG	na	AB080	15.01964°,-24.32666°	29/12/2013	11:14	3	cinzento/verde	fêmea	0	2	ausente
76	RO_OX	A000112	FG	na	AB080	15.01964°,-24.32666°	29/12/2013	12:06	4	castanho	fêmea	0	2	4

Captura #	Anilhas de cor	Anilha de metal	Ilha	Zona	Local	Coordenadas Google Earth	Data	Hora	Idade	Cor da íris	Sexo	Gordura	Músculo	Pelada de incubação
77	BR_RX	A000114	FG	na	AB081	15.01776°,-24.3267°	29/12/2013	14:00	4	castanho	macho	0	3	ausente
78	BR_BX	A000116	FG	na	AB076B	15.02026°,-24.32497°	30/12/2013	08:55	4	castanho	macho	0	2	ausente
79	BR_WX	A000117	FG	na	AB082	15.01558°,-24.32261°	30/12/2013	12:54	4	vermelho	macho	0	2	ausente
80	BR_YX	A000118	FG	na	AB084	15.01495°,-24.32501°	31/12/2013	10:27	4	castanho	macho	0	2	5
81	RR_XR	A000119	SN	na	AB088	16.63766°,-24.35239°	07/01/2014	09:30	6	vermelho	macho	0	3	ausente
82	RR_XB	A000121	SN	na	AB089	16.63783°,-24.35187°	07/01/2014	11:08	6	vermelho	macho	1	2	ausente
83	BB_XB	A000122	SN	na	AB089	16.63783°,-24.35187°	07/01/2014	11:17	5	verde	fêmea	0	2	ausente
84	RR_XY	A000123	SN	na	AB087B	16.62915°,-24.3503°	07/01/2014	16:17	6	castanho	macho	1	2	ausente
85	RR_XO	A000125	SN	na	AB090	16.63747°,-24.35427°	08/01/2014	10:10	6	castanho	macho	0	2	ausente
86	RB_XR	A000126	SN	na	AB090	16.63747°,-24.35427°	08/01/2014	10:34	5	verde	macho	0	2	ausente
87	RB_XB	A000127	SN	na	AB091	16.63703°,-24.35454°	08/01/2014	11:35	6	vermelho	macho	0	2	ausente
88	RB_XW	A000128	SN	na	AB092	16.62563°,-24.3311°	09/01/2014	10:22	6	vermelho	macho	0	3	ausente
89	RB_XY	A000129	SN	na	AB093	16.62645°,-24.33021°	09/01/2014	12:40	6	vermelho	macho	1	2	ausente
90	RB_XO	A000130	SN	na	AB095B	16.63271°,-24.32205°	11/01/2014	13:28	6	castanho	fêmea	0	3	ausente
91	RW_XR	A000131	SN	na	AB095B	16.63271°,-24.32205°	11/01/2014	13:19	6	castanho/verde	macho	0	3	ausente
92	RW_XB	A000132	SN	na	AB097	16.63174°,-24.31114°	14/01/2014	11:34	6	castanho	macho	0	2	ausente
93	RW_XW	A000136	SN	na	AB100	16.63596°,-24.35298°	16/01/2014	11:54	6	vermelho	macho	0	2	ausente
94	RW_XY	A000137	SN	na	AB086B	16.64976°,-24.36057°	17/01/2014	11:00	6	vermelho	macho	1	3	ausente
95	RW_XO	A000138	SN	na	AB101	16.64311°,-24.34414°	18/01/2014	14:01	6	vermelho	fêmea	1	2	ausente

Table XII - Dados de felosas de Cabo Verde *Acrocephalus brevipennis* capturadas: anilhas, médias das biometrias, muda, parasitas e peso; na = dados não registados.

Captura #	Anilhas de cor	Anilha de metal	Asa	Tarso	Cabeça e bico	Bico até narina	Profundidade do bico	Largura do bico	Código da muda	Primárias novas	Secundárias novas	Parasitas das penas	Peso
1	RX_RR	A000002	66.0	na	na	na	na	na	O	na	na	na	17.2
2	RX_RB	A000005	64.0	25.5	37.3	9.4	2.4	4.3	O	na	na	na	16.2
3	RX_RW	A000011	60.5	25.3	36.6	8.2	2.5	3.8	O	na	na	na	16.5
4	RX_RY	A000012	61.5	25.6	35.5	9.4	3.2	5.2	P	na	na	na	14.9
5	RX_BR	A000016	65.0	28.9	39.0	11.8	3.7	5.4	M	na	na	na	18.2
6	RX_RO	A000017	67.0	28.2	39.0	11.2	3.7	5.5	O	na	na	na	18.3
7	RX_BB	A000018	63.0	25.5	37.3	11.5	3.4	5.0	O	na	na	na	14.8
8	RX_BW	A000019	67.0	26.9	38.5	12.4	3.9	5.7	O	na	na	na	17
9	RX_BY	A000023	62.0	26.7	37.7	11.2	3.7	5.6	O	na	na	na	16
10	RX_BO	A000025	66.0	27.2	39.4	11.3	3.7	5.4	O	na	na	na	18.4
11	RX_WR	A000027	62.0	27.6	38.4	11.3	3.6	5.1	O	na	na	A	17.5
12	RX_WB	A000028	67.0	28.5	40.7	12.1	3.5	5.3	O	na	na	F	18.4
13	RX_WW	A000029	63.0	27.6	39.3	11.7	3.6	5.6	M	na	na	F	18.9
14	RX_WY	A000030	60.0	24.8	38.9	11.2	3.6	4.8	M	na	na	F	14
15	RX_WO	A000031	66.0	27.2	41.0	12.4	3.7	5.9	M	na	na	F	19.2
16	RX_YR	A000032	61.0	24.2	37.9	10.9	3.5	5.2	M	na	na	F	15.2
17	RX_YB	A000034	63.0	25.9	37.1	10.5	3.2	5.2	O	na	na	C	14
18	RX_YW	A000035	63.0	25.9	37.5	11.1	3.6	5.3	O	na	na	A	16.6
19	RX_YY	A000036	65.5	28.4	40.6	12.1	3.7	5.6	O	na	na	D	19.1
20	RX_YO	A000038	65.0	27.5	39.7	12.2	3.5	5.4	O	na	na	F	18
21	RX_OR	A000039	61.0	25.6	38.4	11.4	3.4	5.2	O	na	na	F	17.3
22	RX_OB	A000042	62.0	26.2	38.5	11.3	3.8	5.3	O	na	na	E	17.9
23	RX_OW	A000043	66.0	27.8	41.1	11.4	3.7	5.5	O	na	na	E	19.9
24	RX_OY	A000045	65.0	25.4	37.5	11.7	3.6	5.3	M	na	na	F	16.4
25	RX_OO	A000046	62.0	24.5	37.4	10.4	3.8	5.4	O	na	na	A	16.3

Captura #	Anilhas de cor	Anilha de metal	Asa	Tarso	Cabeça e bico	Bico até narina	Profundidade do bico	Largura do bico	Código da muda	Primárias novas	Secundárias novas	Parasitas das penas	Peso
26	BX_RR	A000047	62.5	24.9	38.7	11.5	3.8	5.4	M	na	na	F	15.7
27	BX_RB	A000048	66.0	28.2	39.4	11.4	3.9	5.7	M	na	na	F	16.9
28	BX_RW	A000050	64.0	27.5	38.4	11.4	3.7	5.3	M	na	na	F	18.3
29	BX_RY	A000051	65.0	26.6	37.7	11.1	3.5	2.8	M	na	na	F	17.2
30	BX_RO	A000054	65.0	27.3	38.0	11.4	3.4	5.6	O	na	na	F	17.6
31	BX_BR	A000055	61.0	26.1	38.3	11.2	3.6	5.3	M	na	na	F	17.1
32	BX_BB	A000056	65.0	28.3	40.1	11.7	3.6	5.6	M	na	na	G	19.3
33	BX_BW	A000057	62.5	25.9	36.2	9.9	3.4	5.2	P	na	na	E	14.6
34	BX_BY	A000058	66.0	28.1	36.9	11.0	3.4	5.8	O	na	na	D	18.8
35	BX_BO	A000059	64.0	25.0	37.6	11.7	3.7	5.4	M	na	na	F	17.6
36	BX_WR	A000061	65.0	28.0	39.2	10.8	3.7	5.5	S	na	na	F	16.3
37	BX_WB	A000062	67.0	25.5	39.4	11.8	3.7	5.2	M	na	na	F	19
38	BX_WW	A000063	63.0	26.6	40.3	13.0	3.6	5.7	O	na	na	F	16.9
39	BX_WY	A000065	63.0	26.8	39.6	11.1	3.6	5.3	O	na	na	F	16.1
40	BX_WO	A000066	65.0	26.7	38.8	11.7	3.8	5.4	S	na	na	F	17.5
41	BX_YR	A000067	63.0	27.3	39.6	11.9	3.8	5.5	S	na	na	F	18.1
42	BX_YB	A000068	63.0	25.5	38.4	11.2	3.6	5.3	S	na	na	F	17
43	BX_YW	A000070	64.5	27.1	39.1	11.5	3.4	5.2	M	na	na	F	na
44	BX_YY	A000073	67.0	27.8	38.4	11.3	3.5	5.1	O	na	na	B	18.2
45	BX_YO	A000074	67.0	27.0	38.1	10.6	3.5	4.9	O	na	na	A	18.4
46	BX_OR	A000076	63.0	27.0	38.7	10.9	3.6	5.8	O	na	na	A	16.8
47	BX_OB	A000077	65.0	28.5	39.5	11.2	3.6	5.6	O	na	na	D	17.9
48	BX_OW	A000080	62.0	25.0	37.3	10.9	3.4	5.3	O	na	na	D	16.6
49	BX_OY	A000081	67.0	27.9	39.7	11.7	3.7	5.4	O	na	na	A	18.3
50	BX_OO	A000082	63.0	26.2	37.3	10.6	3.6	5.3	O	na	na	A	18.7
51	RR_RX	A000085	64.0	27.1	39.1	11.7	3.7	5.4	M	na	na	F	17.6
52	RR_BX	A000086	66.0	26.9	40.4	12.3	3.9	5.3	M	na	na	F	17.9

Captura #	Anilhas de cor	Anilha de metal	Asa	Tarso	Cabeça e bico	Bico até narina	Profundidade do bico	Largura do bico	Código da muda	Primárias novas	Secundárias novas	Parasitas das penas	Peso
53	RR_WX	A000087	64.0	27.3	39.8	11.3	3.9	5.2	M	na	na	F	18.6
54	RR_YX	A000088	65.0	26.8	38.1	11.5	3.7	5.3	M	na	na	F	17.3
55	RR_OX	A000089	67.5	27.6	40.6	12.0	3.7	5.6	M	8 a 10	0	F	8.5
56	RB_RX	A000090	65.0	27.9	39.6	11.2	3.8	5.6	M	9, 10	0	E	18.1
57	RB_BX	A000091	67.0	27.7	39.8	11.5	3.7	5.5	M	7 a 10	1 (na asa direita)	F	18.8
58	RB_WX	A000093	64.0	26.1	38.8	11.4	3.7	5.3	M	8 a 10	0	F	16.6
59	RB_YX	A000094	61.0	25.8	38.4	11.9	3.7	5.4	M	8 a 10	0	F	17
60	RB_OX	A000095	64.0	28.4	39.2	11.1	3.8	5.4	M	7 a 10	0	F	18.3
61	RW_RX	A000096	65.0	27.4	39.5	11.2	3.8	5.4	O	0	0	E	17.9
62	RW_BX	A000097	61.0	26.3	38.8	10.6	3.2	5.3	O	0	0	D	17.1
63	RW_WX	A000098	63.0	24.9	39.7	11.4	3.7	5.5	M	5 a 10	0	D	18.2
64	RW_YX	A000099	64.0	27.3	39.6	11.2	3.6	5.4	M	7 a 10	0	F	18.5
65	RW_OX	A000100	64.0	25.5	39.5	11.5	3.7	5.5	M	7 a 10	0	F	19
66	RY_RX	A000101	66.5	25.7	39.5	11.7	3.7	5.3	M	6 a 10	1	F	18.5
67	RY_BX	A000102	63.0	25.4	37.1	10.1	3.7	5.1	M	4 a 10	1 e 2	F	15.3
68	removed	A000103	65.0	26.2	38.9	11.7	3.5	5.0	M	7	0	F	17.6
69	RY_WX	A000104	64.0	26.3	38.8	11.1	3.6	5.6	M	6 a 10	S1 caida	F	16.8
70	RY_YX	A000105	61.0	27.5	38.4	10.7	3.6	4.8	M	7 a 10	0	F	17.7
71	RY_OX	A000106	63.0	26.3	39.1	11.5	3.5	5.4	M	8 a 10	0	F	18.7
72	RO_RX	A000107	70.0	27.6	41.1	11.5	3.9	5.8	M	6 a 10	1	F	19.7
73	RO_BX	A000109	67.0	25.8	39.9	10.8	3.5	5.6	M	6 a 10	1	F	18.9
74	RO_WX	A000110	67.0	25.8	39.0	11.2	3.8	5.3	M	7 a 10	0	F	16.8
75	RO_YX	A000111	64.0	27.3	38.2	10.6	3.7	5.3	O	0	0	E	15.6
76	RO_OX	A000112	62.0	25.7	37.4	10.3	3.5	5.0	M	8 a 10	0	E	14.9
77	BR_RX	A000114	65.0	27.0	39.6	11.5	3.8	5.4	B	0	0	F	19
78	BR_BX	A000116	66.0	28.6	39.1	11.2	3.5	5.3	M	8 a 10	0	E	19.1
79	BR_WX	A000117	67.0	27.7	39.9	11.9	3.7	5.5	M	8 a 10	0	F	18.6

Captura #	Anilhas de cor	Anilha de metal	Asa	Tarso	Cabeça e bico	Bico até narina	Profundidade do bico	Largura do bico	Código da muda	Primárias novas	Secundárias novas	Parasitas das penas	Peso
80	BR_YX	A000118	63.5	26.5	39.6	12.0	3.4	5.4	M	6 a 10	0	F	16.8
81	RR_XR	A000119	65.0	26.6	40.8	11.9	3.7	5.7	M	0	0	C	20.8
82	RR_XB	A000121	65.0	25.7	40.8	12.6	3.8	5.5	M	8 a 10	0	F	20.1
83	BB_XB	A000122	63.0	24.9	37.7	10.0	3.4	5.1	P	0	0	A	17.3
84	RR_XY	A000123	65.5	26.0	39.6	11.7	3.8	5.2	M	7 a 10	0	A	18.4
85	RR_XO	A000125	66.0	26.1	39.6	11.8	3.8	5.6	M	6 a 10	0	F	19.5
86	RB_XR	A000126	63.0	26.2	39.3	12.2	3.6	5.4	P	0	0	A	na
87	RB_XB	A000127	65.0	27.1	39.9	12.5	3.5	5.5	S	0	0	F	18.2
88	RB_XW	A000128	64.0	26.9	40.2	12.4	3.5	5.5	M	5 a 10	1 a 3	A	18.6
89	RB_XY	A000129	64.0	26.7	40.4	12.5	3.6	5.1	O	0	0	E	na
90	RB_XO	A000130	63.0	24.2	39.7	11.6	3.4	5.2	E	todas	todas	F	15.6
91	RW_XR	A000131	67.0	25.2	39.2	11.9	3.5	5.4	E	todas	todas	E	15.8
92	RW_XB	A000132	64.0	26.9	40.9	12.4	3.7	5.2	M	5 a 10	1 e 2	E	17.1
93	RW_XW	A000136	63.0	26.5	40.8	12.7	3.7	5.6	M	3 a 10	1 e 2	F	18.8
94	RW_XY	A000137	66.0	27.1	40.3	12.5	3.6	5.0	M	todas	todas	F	18.6
95	RW_XO	A000138	63.0	24.5	38.9	11.7	3.6	5.3	M	5 a 10	1 e 2	F	16.6

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