CRYPTIC INTRODUCTIONS AND GEOGRAPHICAL PATTERNS IN BIRD COLOR: IMPLICATIONS FOR THE STUDY OF EVOLUTIONARY DIVERGENCE

by

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ABSTRACT OF THE DISSERTATION

Cryptic introductions and geographical patterns in bird color: implications for the study of evolutionary divergence

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In my dissertation I focused on several topics, two of which involve islands and another that follows logically from my work on islands: contemporary evolution and cryptic introductions, variation in island plumage coloration, and variation in plumage coloration within a lineage. I chose to work with the eastern bluebird (*Sialia sialis*) because it has an isolated island subspecies long thought to be endemic to Bermuda based upon striking plumage differences. In my first chapter I used microsatellite data to explore the origin and current status of this island population. Through my analysis I determined that the Bermuda subspecies represents one of the few known cases of a vertebrate cryptic invader that was likely introduced by humans approximately 400 years ago. Oceanic islands have a relatively recent history of human colonization and in the absence of paleontological or molecular evidence it should not be assumed that island species are native.

In both my second and third chapters I used avian perceptual modeling to deconstruct blue plumage coloration into four components (hue, chroma, percent UV, and brightness). In my second chapter, I used feathers from live birds to detail how color

varied between island and mainland populations. I found significant differences in hue (shorter wavelengths) and increased brightness on Bermuda; showing rapid change over a short time frame. My work suggests that we need to define better what constitutes a 'native' species, especially in cases such as the eastern bluebird in Bermuda where there has been sufficient divergence in morphology to be classified as a subspecies.

My third chapter used museum specimens across the bluebird range to attribute specific color components to detectable differences between subspecies. Differences in percent UV and chroma accounted for most intraspecific variation. These components are thought to be condition dependent and may signify the importance of individual-level variation in phenotypic evolution. Furthermore, individual components did not vary in a coordinated manner, implying modularity between the mechanisms controlling color expression.

Acknowledgement and/or Dedication

First and foremost I want to thank Julie Lockwood, my advisor throughout this process. More than anyone else, she has helped me become a better scientist and to explore the 'geeky' ideas in life. I cannot imagine a better advisor and colleague and I am grateful for finding a home in her lab at Rutgers. I am also indebted to my committee members for more help and advice than they probably realize they were dispensing. I value my interactions with each of them. Dina Fonseca's commitment and no nonsense attitude helped me overcome some serious deficiencies in population genetics. Peter Morin's generosity and bluntness has helped me in ways that I am still realizing. I have learned more about science in general through my interactions with him and will always have a model of the well-rounded scientist to emulate. Phill Cassey led by example to get me started on animal coloration, and without his tolerance I wouldn't be so excited about the direction my research has taken. Finally, Peter Smouse has been a keen mentor helping me keep ideas and concepts in perspective. He has freely given me all the advice and perspective I've needed, whenever needed, and I greatly appreciate his contributions.

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with no preconceptions for the eventual outcome and I am acutely aware of the loving and generous role you've played in my life.

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INTRODUCTION

Islands are an important source of information on evolutionary processes and the mechanisms underlying the generation of biodiversity. Early work by Mayr (1964), MacArthur and Wilson (1967), Lack (1976), and Grant (1965), to name just a few, set the stage for a rich exploration of island faunas that continues to yield important insights to this day. These insights range from patterns in community assembly and invasion biology to phenotypic divergence in animal coloration. Islands also feature heavily in studies of evolutionary convergence. The study of avian biology on islands has a long history (Blackburn *et al.* 2009; Lack 1976; Mayr 1964) and birds have figured prominently in research on speciation rates (Moyle *et al.* 2009), the taxon cycle (Kimura *et al.* 2002; Ricklefs& Bermingham 2007), and contemporary evolution (Blondel 2000; Postma& Gienapp 2009; Zink *et al.* 2005). Avian plumage coloration on islands has an especially prominent role in this literature (Doucet *et al.* 2004; Driskell *et al.* 2010; Grant 2001). This focus on insular plumage variation is due in part to the fact that islands represent discrete units and their inhabitants typically do not exhibit clinal variation in coloration.

For my dissertation I chose to work with the eastern bluebird (*Sialia sialis*), a charismatic and wide-ranging species found throughout North and Central America. They are an ideal species to study for several reasons. First, eastern bluebirds have been an important model for research into life history trait and plumage variation. This research has yielded an incredible trove of information, including but certainly not limited to insights into extra-pair copulations, competition, mate selection, and intraspecific variation in plumage coloration (Gowaty& Plissner 1998; Siefferman& Hill 2003). This body of work facilitates the formation of hypotheses and provides a starting

point for evolutionary questions. Another reason I chose to work with eastern bluebirds is because they can be easily manipulated and studied. Eastern bluebirds are obligate cavity nesters and will readily use nest-boxes erected by humans in a wide range of habitats (Gowaty& Plissner 1998). Finally, there are at least eight subspecies of the eastern bluebird, and one of them (*S. s. bermudensis*) is located on the island of Bermuda. Bermuda is situated in the North Atlantic approximately 1000km southeast of Cape Hatteras, North Carolina. This population, along with the vast literature on virtually all aspects of bluebird behavior and reproduction, is the perfect confluence of attributes for studies of ecology and evolution, and provided the primary reason I became enamored with this species.

The Bermuda population of bluebirds is interesting for two reasons: cryptic origins and casual observations of bluer blue. I will first discuss the uncertainty surrounding the provenance of bluebirds on Bermuda. Bermuda was colonized by the British in the early 1600s, and at that point drastic habitat alteration took place as land was cleared for timber and agriculture (Verrill 1902). Bluebirds were first mentioned as resident on Bermuda in the literature in the mid-1800s, at which time they were noted as being abundant (Jones 1859). Bluebirds had always been assumed to be endemic to Bermuda (Verrill 1901), however, contemporary work has found no evidence of bluebirds from the island's subfossil record (Olson *et al.* 2005). Furthermore, open habitat required for foraging did not exist prior to colonization of the islands. There are also anecdotal reports of birds (northern cardinals, *Cardinalis cardinalis*) being shipped from Bermuda to Europe or brought into the islands for commerce (Verrill 1901).

The second characteristic that makes the Bermuda population interesting is past observations in the literature commenting upon the 'bluer' or 'brighter' plumage coloration (Bradlee *et al.* 1931; Prentiss 1896; Webster 1973). It is unusual for an insular species to exhibit increased coloration (Grant 2001; Price 2008), and I immediately became interested in what component of the color signal was changing to create a perception of increased ornamentation.

Before an investigation of plumage differences could be initiated, there were several questions regarding the current status of Bermuda's bluebirds that needed to be addressed. Findings from a population-level analysis could impact the manner in which color differences were analyzed and interpreted. Tantamount to understanding how color changes on the island is the relationship of bluebirds on Bermuda to the mainland. Where did the founding individuals originate and how old and connected to the mainland is this population? Without this information it would be hard to determine the scope and direction of plumage change on the island.

In Chapter One titled "Cryptic invasion and the interpretation of island biodiversity" I present a thorough treatment of the history and current status of this island population. I use microsatellite loci collected from individuals in Bermuda and two geographic regions in eastern North America to infer current genetic connectivity and the probable source populations for the Bermuda population. I explore several introduction scenarios that differ with regard to the age of the population and role that humans played in the bluebird's settlement of Bermuda. I found that bluebirds appear to have been introduced by humans sometime between 1600 and 1850 based upon several compelling lines of evidence. In addition, I found no evidence for ongoing gene flow. This means

that birds on Bermuda have had a relatively brief history on the island and that any subsequent changes in appearance happened quickly in isolation.

Using insight gained from the first chapter of my dissertation, I then began an analysis of how plumage differs in the Bermuda bluebird population as compared to its putative source population in eastern North America. There are several reasons why increased ornamentation (e.g., a brighter plumage) is unusual in island birds. First, sexual dichromatism tends to be reduced on islands, with reductions in male ornamentation and increases in female ornamentation contributing to a net decrease in contrast between the sexes (Price 2008). Second, a number of papers describe instances where island species show overall reductions in contrast or ornamentation (Grant 1965; Grant 2001; Olson 1994; Omland 1997). This all contrasts with the 'bluer' plumage Bermuda bluebirds described above. If birds appear 'brighter' or 'bluer' on Bermuda, then those are traits that have increased in ornamentation.

The Bermuda bluebird population is ideal for testing broad patterns in island plumage evolution because of the extensive research describing how color components vary with measures of individual quality and fitness (Siefferman& Hill 2005a; Siefferman& Hill 2005b). The blue plumage of bluebirds is produced by the arrangement of feather microstructures that scatter light in a coherent manner (Shawkey *et al.* 2005). Blue color in this sexually dichromatic species is condition dependent and components of the color signal are correlated with measures of fitness, parental care, and mate quality ((Siefferman& Hill 2003, 2005a) c.f. (Peters *et al.* 2011)). Some parts of the color signal are under genetic control while others have been linked to individual measures of

condition. With this knowledge I could develop expectations for the direction and magnitude of plumage change and then test them with bluebirds from Bermuda.

My second chapter on island plumage, “Rapid evolution of ornamented plumage and increased sexual dichromatism in an island bird”, investigates differences in color signal components between island and mainland populations of eastern bluebirds. In this chapter I approach coloration with a nuanced appreciation for how individual components of the color signal vary across regions. Based on recent technological advances, biologists can objectively deconstruct color expression into two parts; the chromatic and achromatic signals. The chromatic signal consists of three components; hue, chroma and ultra-violet. Hue is the technical term for color, chroma is a measure of a hue’s purity in the human-visible spectrum (400 – 700nm), and ultra-violet reflectance (percent UV) is a measure of the light reflected between 300 and 400nm. The achromatic signal, called brightness, is psychologically processed by vertebrates independent of the chromatic signal. Using these measures of color’s constituent parts, I determined if birds from Bermuda exhibited differences as compared to mainland populaitons and if so, how was it manifested in the plumage? In general, bluebirds exhibited increased ornamentation on Bermuda, and sexual dichromatism was also increased relative to the mainland. This pattern held for both sexes. I found the color components hue and brightness to differ the most between island and mainland birds.

My work on island plumage variation showed that specific components of the color signal differ between island and mainland regions. Why didn’t all of them contribute to differences in the blue coloration? This led to a strong interest in how specific color components vary across the entire geographical range of species. Do the

same components vary across all bluebird subspecies, or is there variation in which traits diverge? My third chapter, “Differential contribution of color signals to avian plumage diversity” follows logically from my initial exploration of color component variation on an island. In the second chapter I was asking the question “how do color components vary on an island and do they behave similarly to color component variation on the mainland.” In the third chapter I take the novel approach of quantifying how each component of the color signal varies across multiple subspecies of eastern bluebirds. I then determine the relative contribution of each color component to the plumage differentiation of subspecies. This is the first time a color signal has been deconstructed and analyzed across subspecies to determine which components contribute the most to plumage color differences between groups. To do this, I use data collected from museum specimens to quantify variation among seven (of the eight) subspecies of eastern bluebird.

There are several important reasons why I chose to study variation in color components across subspecies. While working through the literature on intraspecific variation in color, I realized that almost all research had been conducted at a local-scale, primarily focusing on individuals within a single population (Hill 2006; Johnsen *et al.* 2003; Laczi *et al.* 2011; Shawkey *et al.* 2007; Siefferman& Hill 2005a). I had already studied variation within a relatively young population and contrasted it with the parent population from which it was founded. This result illustrated the possibility of differential expression in components of the color signal, which is a pattern rarely discussed in the literature. I then realized that there could not be a significant discussion of how color varies across taxonomic groups without an improved understanding for how the

components of a signal contribute to these differences. For instance, do changes in hue contribute most to changes in phenotype or does percent UV play a larger role?

Several exciting patterns became evident from the analysis in chapter three. First, percent UV played the largest role in differences between subspecies. After percent UV was chroma, followed by hue and then brightness with the smallest contribution to subspecies differentiation. My results show that across taxa, percent UV and chroma may be important determinants of phenotypic divergence.

Each chapter was written with my advisor, Dr. Julie Lockwood as a stand-alone manuscript formatted for the journal to which it has been submitted. Chapter one is formatted for *Molecular Ecology* and was co-written with Dina Fonseca. Chapter two is formatted for *Proceedings of the Royal Society B: Biological Sciences* with Phill Cassey, and chapter three is formatted for *Plos One* and will be submitted there.

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CHAPTER 1

Cryptic Introductions and the Interpretation of Island Biodiversity

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Abstract

Species with cryptic origins (i.e. those that cannot be reliably classed as native or non-native) present a particular challenge to our understanding of the generation and maintenance of biodiversity. Such species may be especially common on islands given that some islands have had a relatively recent history of human colonization. It is likely that select island species considered native may have achieved their current distributions via direct or indirect human actions. As an example, we explore the origins of eastern bluebirds (*Sialia sialis bermudensis*) on the island of Bermuda. Considered native to the island and a distinct subspecies, this population has diverged in morphology relative to mainland North America. Using microsatellite markers and several other lines of evidence, we show that the Bermuda population of bluebirds is the likely result of a single human-assisted colonization event that occurred during the 1600s; making this species a cryptic invader. To our knowledge this is the youngest example of a terrestrial vertebrate cryptic invader and the only one designated as a subspecies. We suggest that the eastern bluebird is not an isolated case of cryptic invader either on Bermuda or elsewhere and that extreme caution be exercised when studying present-day distributions of organisms. Such cases require biologists to reconsider their definitions of native and non-native species.

Introduction

A peculiarity of biodiversity research is the often implicit assumption that the first recorded (usually European) list of species occupying a locale consists of only native taxa (Carlton& Geller 1993). We tend to discount the possibility that species were introduced as exotics (accidentally or on purpose) via human transportation mechanisms that existed prior to, or during, the early phases of the Age of Exploration (15^{th} to 17^{th} centuries). This assumption has been proven wrong on several occasions (e.g. Wilmshurst *et al.* 2008; Yan *et al.* 2001), but there persists a deficit in our knowledge of the origins of many species that may reasonably be considered cryptogenic (i.e., of unknown origin, (Carlton& Geller 1993)). To date, most research on cryptogenic species has either centered on cryptic introductions of genetically novel individuals (e.g. Saltonstall 2002), or on species that have unusually broad distributions that may have been achieved via cryptic introduction events (e.g. Blakeslee *et al.* 2008; McGlashan *et al.* 2008). Using the origin of eastern bluebirds (*Sialia sialis*) on the island of Bermuda, we highlight the pitfalls associated with assuming native status in the absence of molecular and paleontological evidence, especially relative to understanding the origins and maintenance of island biodiversity.

A standard metric used in island biogeography is the number of taxa present on one or more islands (Macarthur& Wilson 1967). This metric is then analyzed in terms of how a suite of factors influence it, such as geographic isolation, island size, and within-island habitat diversity. Such analyses have provided a basic foundation for our understanding of colonization, extinction, community assembly, and adaptive evolution

(Whittaker& Fernandez-Palacios 2007). For the vast majority of cases, systematists working before the advent of molecular methods determined the taxonomic identities of these species based on morphology alone. There is no doubt that some cryptic species remain to be 'discovered' through the use of molecular and morphological methods, and this certainly will alter components of island biogeography (e.g. Lohman *et al.* 2010). We suggest that it is also likely that some of these taxa represent populations that colonized an island via the direct or indirect action of humans, and are thus cryptic invaders.

Invaders that have become established since modern records (post-1800s) are generally identified as non-natives and are thus not considered in most island biogeographical studies. In contrast, cryptic invaders can become established as far back as 1000 years ago, and they are almost universally and naively identified as natives. Mistakenly attributing native status to such species may artificially inflate estimates of island biodiversity. This issue comes to the fore in instances where cryptic invaders evolved substantial life history or morphological features since their time of founding. Is the presence of these species consistent with the theories embodied in island biogeography, or are they something wholly unique that require us to reconsider some of our basic assumptions about the origin and maintenance of island biodiversity? We provide evidence that the eastern bluebird population on the island of Bermuda sits precisely in this gray area, and we describe how the presence of such species (more broadly) challenges our existing framework for understanding island biodiversity.

History of Bermuda and its Songbirds

Bermuda is a collection of over 180 islands covering 53 km^2 that lies in the North Atlantic Ocean, with three islands large enough to hold substantial numbers of people. It is commonly classed among other Caribbean islands, and to a large extent aligns itself culturally with these islands today; however it lies well north of the Caribbean Ocean (Fig. 1). The nearest landfall is just over 1000km south-southeast at Cape Hatteras, North Carolina, USA. The islands are situated at a strategic location in the Atlantic, which made it a sometime replenishment spot for Spanish and Portuguese ships that sailed this stretch of ocean in the 1500s, and later rendered it a key colonization outpost for the British in the early 1600s (Craven 1937).

Bermuda's colonization history is quite colorful as it was initiated by the wreck of a relief ship sent to reinforce the Jamestown Colony in Virginia, USA, in 1609. Unlike many other oceanic islands, Bermuda does not seem to have been inhabited by humans before the first permanent British settlements. It was not long after these early arrivals, however, that the flora and fauna began to undergo permanent alteration. The purposeful and accidental importation of plants and animals began almost immediately after initial settlement, with several of these species quickly attaining pest status (Verrill 1902). Wild pigs were present on the island for at least 100 years prior to 1612, having likely been placed there by pirates as a ready food source (Verrill 1902). Cats and rats were quite common in the 1600s and may have been introduced prior to permanent human settlement as well (Verrill 1902). The English colonists began at once to cut the abundant Bermuda cedar (*Juniperus bermudiana*) and other native trees for the construction of

dwellings and fortifications, largely deforesting the islands within a span of a few decades (Verrill 1902).

The written and fossil history of the islands suggests a substantial extinction event occurred shortly after the establishment of these permanent human settlements, if not initiated before with the introduction of cats, rats and pigs by itinerant visitors. A suite of endemic passerines became extinct around the 1600s or shortly thereafter (S. Olson pers. comm.). Today there are only 10 passerines that are year-round residents, and of these, only three are considered native (Lockwood& Moulton 1994). The majority of the known-exotic birds were introduced between the years 1800 and 1970, with the one exception being the northern cardinal (*Cardinalis cardinalis*), which was thought to have been released by early inhabitants of the island in their trade with the Virginia Colony (Verrill 1902). Northern cardinals are abundant natives to the North American continent.

The three passerines currently considered native are the eastern bluebird, gray catbird (*Dumetella carolinensis*) and white-eyed vireo (*Vireo griseus*). There are no records, going back 400,000 years, of these three species as fossils (Olson *et al.* 2005) and they are not explicitly mentioned in the accounts of the birds given by early colonists (Verrill 1902). All are native to North America, and none have breeding populations on oceanic islands other than Bermuda, including near-shore islands in close proximity to North America. They cannot clearly be ascribed as survivors of the extinction event, but were not obviously introduced either. This evidence suggests these three species may have been undocumented introductions from the mainland (i.e. they have a cryptic origin). The evidence of northern cardinals being released on Bermuda by the early

colonists (1600s), and the general uptick in the number and range of species introduced during this time period, suggests that purposeful introductions of these species in the 1600s or early 1700s is not out of the question. The counter-evidence for at least two of these native passerines (eastern bluebird and white-eye vireo) is that they have been classified as subspecies based on morphological and plumage differences between the island and mainland populations.

The Bermuda bluebird population was ascribed subspecies status as early as 1901 (Bradlee *et al.* 1931; Gowaty& Plissner 1998; Phillips 1991; Verrill 1901a, b), based upon general impressions of size and color. They were thought to be brighter blue dorsally and deeper red ventrally as well as larger in size on the island. Prentiss (1896) suggested that bluebird song also differed between island and mainland populations. The differences in plumage coloration have received subsequent detailed investigation (Phillips 1991) and Gowaty & Plissner (1998) continue to consider them as a distinct subspecies. We conducted an in-depth analysis of the structural blue coloration of island and mainland bluebirds and found a surprising degree of plumage change in both male and female island birds (Avery et. al. unpublished data). Sexual dichromatism and brightness was elevated whereas hue was shifted towards shorter wavelengths. Females also exhibited less ultraviolet feather reflectance than mainland birds.

If the eastern bluebird colonized the islands via human actions in the 1600s, or if it self-established during this time due to human-initiated changes toward habitat that favored the species, it must have evolved sufficient phenotypic differences to have been classed as an endemic subspecies over this same interval. Although post-invasion

evolution is certainly common, this would represent only the second (Ricklefs& Bermingham 2008), albeit much younger, example of a cryptogenic species having accumulated differences to the extent that it could legitimately be considered a subspecies (see also Johnston& Selander 1964).

Colonization Scenarios

In an effort to untangle the evidence of the origin of Bermuda bluebirds, we evaluated different colonization scenarios using molecular markers. Previous analyses of deeper history have shown no differentiation between North American and Bermudan populations of eastern bluebirds in mitochondrial DNA (R. Fleischer pers. comm.), which indicates either incomplete lineage sorting or contemporary gene flow (Avise 2004). In order to understand more recent historical patterns we evaluated our colonization hypotheses using differences in microsatellite markers, which evolve more rapidly than mitochondrial DNA and can uncover evidence of more recent divergence (Avise 2004).

Based on the history of the island and its passerine fauna, we propose three colonization scenarios. The first is the *Pre-Colonization Scenario* (prior to 1610) where we assume bluebirds were present on Bermuda well before human colonization of the islands, but were rare enough in the early colonial years that writers failed to mention them. We can include under this scenario the possibility that the Bermuda bluebird population expanded considerably after the clearing of forests for agriculture created open habitats within a matrix of forests, which is the preferred habitat of bluebirds. Under this scenario we would expect the Bermuda population to have diverged significantly and

exhibit numerous private alleles reflecting the relatively long isolation between mainland and island populations. We also expect to detect few lingering signs of a population bottleneck as allele frequencies are expected to stabilize over the long time periods assumed in this scenario, and because of the possibility of a population expansion after European colonization.

The second hypothesis is the ***Recurring Natural Scenario*** (1610 to present) in which we posit that naturally occurring migrants colonized the island when bluebird habitat was created by land clearing for agriculture and that they continue to exchange genes with occasional migrants. Eastern bluebirds are considered facultative migrants, and different populations exhibit different migratory strategies (sedentary to highly migratory) based upon climatic variation (Gowaty& Plissner 1998). Observations from Bermuda have noted large, high-flying flocks of bluebirds in the company of cedar waxwings (*Bombycilla cedrorum*; D. Wingate pers. comm.) (Bangs& Bradlee 1901; Bradlee *et al.* 1931), and it has been surmised that birds from the mainland are making landfall on the island during their Fall migration south (Bradlee *et al.* 1931). Bermuda provides refuge to many species during migration and a substantial number over-winter (JDA pers. obs., Bermuda Audubon, Christmas Bird Count Data). It is not isolated like the Hawaiian islands, especially to those species that winter in the Caribbean or South America. These migratory populations provide the putative source for the initial inoculation of the island with individual bluebirds that became a resident group. Under this scenario, we expect few or no private alleles reflecting recent isolation and continuing gene flow. There is no reason to suspect that the occasional influx of new

individuals into Bermuda has waned through time. In addition, we expect to see a broad subset of mainland alleles within the Bermuda population since naturally occurring migrants should effectively sample most alleles present in the mainland, including rarer alleles.

The third hypothesis is the ***Human Introduction Scenario*** (1610 – early 1800s) where we posit that bluebirds were introduced to Bermuda from North America perhaps around the same time as the northern cardinal (early 1600s) but no later than the 1840s when we have written record of bluebirds in large numbers (Jones 1859). Eastern bluebirds have a striking blue plumage and melodious song that early traders may have considered attractive to bird enthusiasts in Britain. The same qualities certainly led other individuals to release Eastern bluebirds in locations as far away from the bird's native range as Tahiti (Long 1981). Under this scenario, we would expect the Bermuda population to have few or no private alleles reflecting their recent isolation, and to have undergone a substantial genetic bottleneck since most exotic birds were released in quantities of under 50 individuals (Blackburn *et al.* 2009). Given that introduced bluebirds should have few competitors for foraging and nesting sites at this time, we suspect that the population size would have been able to increase rapidly, thereby preserving some of the rarer alleles that might be purged in populations that stay small (Nei *et al.* 1975). As a result, we expect moderate levels of allelic diversity with very few rare alleles. We also expect a regional signature in the microsatellite data, indicative of bluebirds being collected locally for transport to Bermuda.

To create an evidence base to test the veracity of each of our colonization scenarios, we collected molecular marker information from eastern bluebirds in Bermuda and across a reasonable swath of the nominate race's (*S. s. sialis*) native range in North America. This subspecies is distributed across all of eastern North America from Florida to Nova Scotia and west to the Rocky Mountains with several subspecies in Mexico and portions of Central America (Gowaty& Plissner 1998; Phillips 1991). The migratory populations are all in the northern latitudes, with some evidence that midwestern birds migrate towards the southeast in Autumn (Gowaty& Plissner 1998). Thus, no matter the colonization scenario, we believe the founding population is likely to have been located along the eastern seaboard or the upper Midwest given early shipping and commerce routes and the fact that migratory behavior is climate dependent.

Methods

Sample collection

We included a total of 114 bluebirds in the analysis from Bermuda and mainland North America (Fig. 1). We initially treated all four mainland sampling localities (New Jersey, North Carolina, Iowa and Minnesota) as separate populations (Fig. 1). Because of the proximity between individuals caught in New Jersey and North Carolina, as well as between Iowa and Minnesota, we first tested for differences in allele frequencies between each pair of localities. No tests performed on the subdivided data supported breaking the data into multiple populations (see below). Therefore, we lumped North Carolina and New Jersey together into one population (coastal) and Iowa and Minnesota into another

population (continental). We feel this represents a biologically relevant grouping due to the geographic distance involved and evidence of separate migratory pathways from individual bird band recoveries (Gowaty& Plissner 1998).

We caught adult birds using mist-nets and removed a small volume of blood (25 - 100 µL) by puncturing the brachial vein with a 27-gauge syringe. We then used microhematocrit capillary tubes to collect blood and deposit onto the storage medium (Campbell 1995). Blood samples were stored on FTA cards (Whatman Bioscience).

Molecular methods

We washed (0.5% SDS and TE) and extracted genomic DNA using the manufacturer's room temperature pH treatment protocol. We genotyped all individuals at 12 microsatellite loci (Sialia2, Sialia6, Sialia8, Sialia11, Sialia15, Sialia18, Sialia22, Sialia27, Sialia28, Sialia30, Sialia36, Sialia37) (Faircloth *et al.* 2006) following the PCR protocol developed by Faircloth et. al. (2006). PCR amplifications were performed in 20 µL volumes. Our thermal touchdown cycle (following the 60 – 49.5°C program) differed from Faircloth et. al. (2006) in the following parameters: 95 °C for 5 min at beginning of routine; 95 °C for 30 s at start of each cycle; 30 s at the highest annealing temperature minus 0.5 °C per cycle; 72 °C for 30 s for a total of 21 cycles; followed by 9 cycles of 95 °C for 30 s; 49.5 °C for 30 s; and 72 °C for 30 s. We visualized PCR products on 1% agarose gels and adjusted the number of extension cycles for different primer pairs to control the overall quantity of product generated for each primer set. All loci were subjected to a routine of 21 and nine cycles except for Sialia11, Sialia18, Sialia28, and

Sialia36, which we subjected to 21 and 10 cycles. Sialia15 underwent a routine of 21 and eight cycles. We multiplexed PCR reactions differentiating between overlapping loci with fluorescent dyes (PET, VIC, FAM) and we manually scored genotypes using Genemapper vers. 3.7.

Statistical methods

We calculated summary statistics and population differentiation (AMOVA) using GENALEX 6.3 (Peakall& Smouse 2006), F_{STAT} (Goudet 1995), and ARLEQUIN 3.11 (Excoffier *et al.* 2005). To account for the effects of genetic variation on F_{ST} values we applied Meirmans correction (F'_{ST}) (Meirmans 2006) and calculated D_{est} (Jost 2008) using SMOGD (Crawford 2010). We used GENEPOP 4.0 (Rousset 2008) to test for linkage disequilibrium and deviations from Hardy-Weinberg (using the parameters 10,000 dememorizations; 100 batches; 5,000 iterations per batch) and applied Bonferroni corrections to control for Type I error. We implemented STRUCTURE 2.3.3 (Pritchard *et al.* 2000) as another tool to detect population differentiation setting the number of clusters (K) from 1 – 5 with 10 iterations for each K, and a burn-in period of 10^4 and 10^5 Markov chain Monte Carlo repetitions. We used both the admixture and no admixture ancestry models with sampling locations as priors when previous runs failed to detect structure in our data. We used Program BOTTLENECK (Piry *et al.* 1999), M P Val (Garza& Williamson 2001), and a graphical method devised by Luikart et. al. (1998) to test for a population bottleneck in the Bermuda population. We ran all three models in BOTTLENECK because our data appears to have some loci conforming to the infinite

and step-wise mutation models, and it is not clear given the information at hand which method is most appropriate (Darvill *et al.* 2010). To test for differences in allelic diversity we ran a one-way ANOVA using SAS software (SAS 2003).

Results

The number of alleles ranged from 4–20 per locus and all loci were polymorphic in all populations except for Sialia18, which was monomorphic in Bermuda (Table S1). Overall allelic richness was significantly reduced in the Bermuda population despite a greater sample size than either the coastal or continental mainland populations (Table S1). Bermuda possessed two novel alleles with a frequency of 0.098 (eight individuals with a single copy) and 0.012 (one individual with a single copy). Private alleles were never found in more than three individuals on the mainland.

Linkage disequilibrium and Hardy-Weinberg equilibrium

We found evidence for linkage disequilibrium between two pairs of loci after Bonferroni correction ($P_{Bonferroni} < 0.0007$). Loci Sialia36 and Sialia8 ($p = 0.00005$, S.E. = 0.000044, switches = 25,049), as well as Sialia37 and Sialia8 (0.000382, S.E. = 0.000204, switches = 31,568), were out of equilibrium only in Bermuda, and did not show linkage disequilibrium in the North American populations for the same loci as described in Faircloth et al. (2006). Thus we suspect they are not physically linked. We found two loci that did not conform to Hardy-Weinberg equilibrium after Bonferroni correction; Sialia27 in the Coastal population and Sialia36 in Bermuda (Table S2). Sialia36 exhibited a

heterozygote excess and Sialia27 exhibited a homozygote excess. Removing these loci from subsequent analyses did not change the overall results with one exception (see below), therefore we retained them in the dataset. When we analyzed each individual sampling locality, the deviation from Hardy-Weinberg in Sialia 27 appears to be driven by the North Carolina samples. We feel that this deviation is probably due to sampling bias since the distribution of alleles does not seem problematic (i.e., the alleles comprising homozygous genotypes are also detected in heterozygous genotypes, albeit not in the expected frequencies).

Population divergence

Our F_{ST} and D_{est} values clearly show divergent allele frequencies in Bermuda relative to the mainland (Table S3) ($F_{2,114} = 8.48$, $P = 0.01$). Standardized F'_{ST} values show that allele frequencies in Bermuda are approximately 32% divergent from the mainland (Table S3). There is no evidence for divergence between mainland populations. Our STRUCTURE results consistently showed support for an island cluster and a mainland cluster. Choosing the no-admixture model with prior location information did not help to resolve sampling localities on the mainland (in ungrouped and grouped form) and failed to detect any individuals that clustered outside of their original mainland or island sampling region (Figure S4).

Prior to lumping the Coastal and Continental populations, when Loci Sialia27 was removed from the dataset, we did find a significant difference in allele frequencies between Iowa/Minnesota and North Carolina using the exact G test in GENEPOP.

However, we did not see any differences with the data structured into Coastal and Continental groupings when loci not in Hardy-Weinberg were removed. All other tests for population differentiation in GENEPOP were consistent with the AMOVA results from GENALEX. F_{IS} values, a measure of inbreeding, were not significantly different in each of the three localities based upon a permutational test in ARLEQUIN.

Bottleneck analysis

We found evidence that some loci appear to exhibit stepwise mutation behavior while others appear to behave in ways expected under the infinite allele model, thus we tested for evidence of a bottleneck under each assumption. Under the Infinite Allele Model (IAM) tests were marginally significant with the sign test ($p = 0.057$, $n = 82$) and Wilcoxon test ($p = 0.073$, $n = 82$) for heterozygote excess expected under a recent bottleneck scenario. The stepwise mutation model (SMM) and two-phase model (TPM) were not significant. We also followed the graphical approach devised by Luikart (1998), and this evidence indicates a shift in the allele frequency distribution between Bermuda and North America, a signal that a population bottleneck has occurred (Fig. 2). Both methods are thought to only be effective at detecting recent bottlenecks from $2N_e$ to $4N_e$ generations (Luikart *et al.* 1998; Piry *et al.* 1999). Using M P Val (Garza& Williamson 2001) we obtained a critical M value of 0.86, which does not indicate a recent bottleneck. In addition, it is apparent that the range of alleles from Bermuda is a subset of the most common alleles from the mainland, another indicator of a founding effect (Fig. 3). Finally, there are four loci in which a single Bermudan allele accounts for more than 80%

of the variability. Taken together, this evidence suggests the Bermudan bluebird population underwent either a moderately narrow genetic bottleneck in the recent past (<100 years) which seems unlikey given our M value, or such a severe bottleneck in the more distant past (>100 years) that the genetic signature is still apparent.

Discussion

If the evolution of substantial morphological and life history differences between populations takes considerable time, then it was perfectly reasonable for the intrepid biologist to assume that a distinct form he found on an island is native to that island. There are two recent observations that challenge this long-held assumption. First, species regularly evolve in contemporary time (less than a few hundred years, (Stockwell *et al.* 2003)), and the resultant divergence in traits between populations can be quite substantial (Vellend *et al.* 2007). Second, the dynamics of human colonization across the globe involve considerable trade in live species, and in some geographical locations (principally islands), human colonization occurred within the last millennium (Fitzpatrick& Keegan 2007; Steadman 2006). Given these observations, the contemporary biologist is confronted with the possibility that the unusual variety of plant or animal he found on an isolated island is either not native to that island, or what biologists generally refer to as 'native' has some intriguing and challenging exceptions.

Our analysis of eastern bluebirds across mainland and island populations illustrates the potential problems that scientists may find lurking in island systems. Despite their unique morphology (Avery et. al. unpublished data), bluebirds on the island

of Bermuda appear to be a recently isolated population based upon four lines of evidence. First, we found a profound lack of *in situ* genetic variation among the individual bluebirds resident on Bermuda. While mutation rates for microsatellites are highly variable, average estimates are around 10^{-3} - 10^{-4} for each locus, per gamete, per generation (Avise 2004; Eggert *et al.* 2004). Thus, if this population had been isolated for thousands of years we would expect to see considerably more than the two novel alleles currently present in the Bermuda population. Second, North American and Bermudan populations show 30% divergence in allele frequencies, which is quite striking in light of the dearth of novel alleles. Third, given the moderate mainland allelic diversity at the locus for which Bermuda has become fixed, it is quite likely that a small number of founding individuals led to fixation in the Bermuda population rather than genetic drift leading to fixation acting over long time periods. Fourth, we observed that common mainland alleles are also the most common island alleles, and that single alleles account for a large proportion of the variability at each locus in Bermuda (e.g., allele 269 at Locus Sialia6 has a frequency of 81%).

Since the Bermuda population appears to be of recent origin and is not present in the subfossil record, we can reject the Pre-Colonization Scenario. The question then becomes did it colonize the island naturally or did early human residents intentionally introduce it? We find little support for the Recurring Natural Scenario due to the presence of strongly divergent allele frequencies and a weak signal of a population bottleneck. We expected a broad distribution of mainland alleles under a natural settlement, however, this seems likely under both scenarios given the lack of

differentiation on the mainland. The bottleneck results and subset of common mainland alleles suggests that the Bermuda population was founded in a single event, that included relatively few individuals, and that this population likely rebounded quickly after introduction and has remained isolated ever since. This data supports the Human Introduction Scenario. The open land created by agriculture and tree clearing at this time would have presented an unexploited resource, allowing rapid population growth. In addition, the lack of genetic differentiation between bluebirds across a wide swath of North America suggests that this species has no problem in making long-distance movements, and can do so regularly enough to homogenize gene frequencies across broad geographical areas. Thus, if bluebirds settled naturally on Bermuda in the 1600s after suitable habitat was created there, we would expect to see ongoing gene flow and an effective halt to the fixation of alleles. Instead, Bermuda bluebirds are monomophorpic at locus Sialia18 while mainland bluebirds have another ~ 40% of their variation comprised of different alleles at this locus; even a small number of migrants should have kept this locus from becoming fixed in Bermuda.

Given our results, the subspecies status of Bermuda bluebirds presents an interesting wrinkle in our interpretation of island biodiversity and its generation. Eastern bluebird morphology is clearly evolving at a faster pace than neutral nuclear markers (Avery et. al. unpublished data) and significantly faster than what is often expected for recent population splits (Dlugosch& Parker 2008; Oyler-McCance *et al.* 2010; Pérez-Emán *et al.* 2010; Pruett& Winker 2010; Stockwell *et al.* 2003). This mismatch between phenotypic and neutral molecular change may be a common by-product of evolution in

response to anthropogenic selection pressures (Stockwell *et al.* 2003), perhaps most commonly for species that are non-native to the region in which they are evolving (Dlugosch& Parker 2008). If the movement of live plants and animals via trade has been part and parcel to the presence of permanent human settlements, there are perhaps many more species like the eastern bluebird that are morphologically unique but of relatively recent human-assisted origin (Grueber& Jamieson 2011; Ricklefs& Bermingham 2008). Notably, our results suggest that the other two passerine species currently considered native to Bermuda, the gray catbird and white-eyed vireo, deserve in-depth investigation as to their origins. The white-eye vireo is also considered an endemic Bermudan subspecies as it shows considerable differentiation in morphology relative to mainland populations, and thus may provide further insight into the generation of diversity on the island.

One of the more obvious geographical regions to search for additional cryptogenic species is within the Pacific Islands. There are several recent examples of cryptic invaders coming from this region (Blakeslee *et al.* 2008; Wilmshurst *et al.* 2008), however, investigations have thus far been circumscribed to only those species with wide distributions and no (stated) morphological or life history divergence. We suggest that there is good reason to delve more deeply into the origination of species that perhaps have evolved island-specific morphologies or life history characteristics. The general expectation is birds will lose some plumage ornamentation and exhibit decreased sexual dichromatism. Extra-pair paternity is also less prevalent on islands (Price 2008). We also suggest that other island systems are worthy of similar investigations. For example,

islands in the Caribbean have experienced human activity for 6,000 – 7,000 years, long enough that contemporary species distributions could have been influenced by humans (Fitzpatrick& Keegan 2007; Lee *et al.* 2007; Wilmshurst *et al.* 2008) (see Olson& Ricklefs 2009; Ricklefs& Bermingham 2008). While many researchers are attempting to highlight cryptic diversity in the form of undescribed species in island habitats (see Lohman *et al.* 2010), we feel that revisiting island populations will undoubtedly uncover more situations of contemporary evolution following human-assisted colonization of even very remote islands. Given the short amount of time it took for eastern bluebirds to diverge in morphology as much as they have (Avery et. al. unpublished data), this will be especially true in cases where there are unexplained gaps in species distributions (Ricklefs& Bermingham 2008).

Finally, our results suggest the need to better define what constitutes a ‘native’ species, especially in cases such as the eastern bluebird on Bermuda where there has been sufficient divergence in morphology to have been classified as a subspecies. Biologists studying contemporary species invasions have judiciously steered well clear of this vexing problem (Cox 2004). We suggest that the case of the eastern bluebird on Bermuda is not an exception, and a closer look at species with cryptic origins will further highlight the need to enter a serious dialogue on the subject (Remsen 2010).

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Author Contributions

JDA designed and carried out the research as well as analyzed the data and wrote the paper. DMF provided laboratory space and helped analyze the data. JLL helped design the research and write the paper. JDA conducts research on contemporary evolution and plumage in island birds and the implications for species conservation. DMF studies the introduction, expansion, and transformation of invasive species, particularly disease vectors. Julie Lockwood conducts research on species invasions and extinctions, and how they combine to reshape biodiversity.

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Figure 1. Sampling localities and sample sizes for eastern bluebirds (*Sialia sialis)* from North America and Bermuda. The native range (shown in gray) of the nominate race (*Sialia sialis sialis)* extends into northern Mexico and Canada. The geographical range of other Mexican and Central American bluebird subspecies is not shown.

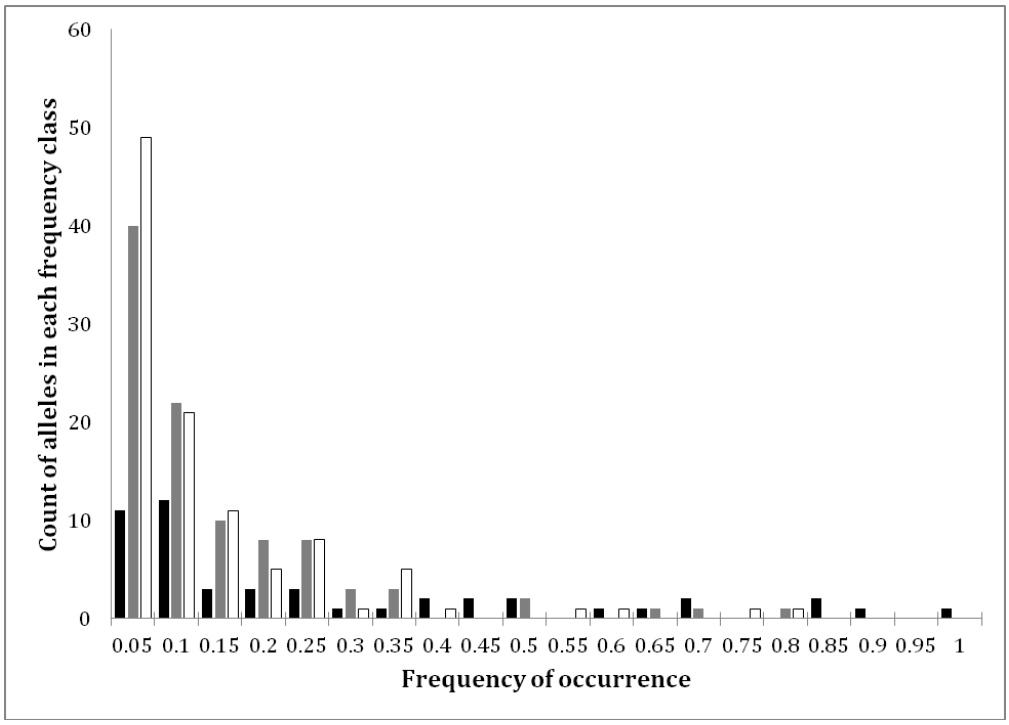


Figure 2. Total allelic counts in 5 % frequency increments for 12 eastern bluebird microsatellite loci sampled from Bermuda (black bars), the inner-continental North American mainland (gray bars), and Atlantic coast of North America (white bars). In non-bottlenecked populations there are many alleles that occur at low frequencies and few alleles that occur with high frequency. Note the longer tail for Bermuda, indicative of more individual alleles that occur with greater frequency. In Bermuda one locus was fixed and two more loci had a maximum of two alleles. Bermuda samples show a characteristic hump shape that signifies a shift towards fewer rare alleles, indicative of a genetic bottleneck.

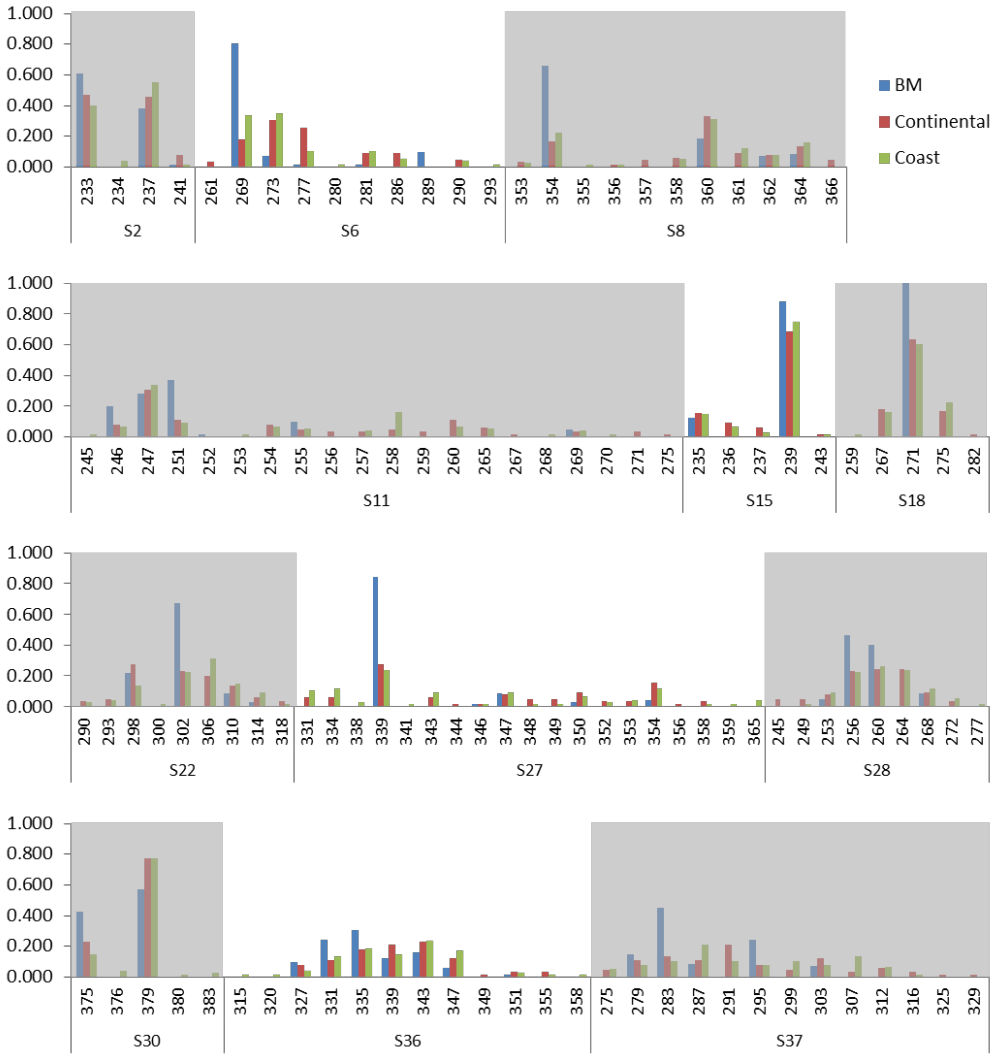


Figure 3. Allele frequencies are depicted across all populations and all loci. Note that Bermuda typically has a subset of the common alleles found across all mainland samples, and that the frequency of these alleles are much greater in Bermuda compared to the mainland. This pattern is characteristic of a recently established population.

Table S1: The number of alleles, effective number of alleles (N_e), allelic richness, and the information index (I) observed per locus across all three sampled regions. N_e is an estimate of the number of equally frequent alleles in an ideal population, allelic richness is number of alleles corrected for sample size, and I, equivalent to the Shannon-Weaver index, is an indicator of allelic and genetic diversity. Mean N_e is approximately twice as large in both the continental and coastal regions than Bermuda ($p = 0.008$; $DF = 2$; $F = 5.56$) but does not differ between mainland sites.

Locus	Bermuda[a]				Continental[b]				Coastal[b]			
	# Alleles	N_e	Diversity	I	# Alleles	N_e	Diversity	I	# Alleles	N_e	Diversity	I
Sialia2	3	1.942	2.805	0.723	3	2.155	3.000	0.873	4	2.310	3.821	0.909
Sialia6	5	1.508	4.610	0.701	7	3.837	7.000	1.577	8	4.745	7.645	1.704
Sialia8	4	2.084	4.000	0.987	10	5.016	10.000	1.800	9	5.542	8.621	1.970
Sialia11	6	3.807	5.804	1.471	15	5.948	15.000	2.160	14	7.212	13.290	2.336
Sialia15	2	1.273	2.000	0.371	5	1.696	5.000	0.821	5	2.000	4.796	0.998
Sialia18	1	1.000	1.000	0.000	4	2.287	4.000	0.992	4	2.146	3.825	0.960

Sialia22	4	1.976	3.964	0.901	8	5.008	8.000	1.795	9	5.236	8.617	1.805
Sialia27	5	1.394	4.762	0.621	15	8.625	15.000	2.422	17	7.696	15.883	2.353
Sialia28	4	2.588	3.999	1.080	8	5.024	8.000	1.746	8	5.312	7.649	1.824
Sialia30	2	1.958	2.000	0.682	2	1.599	2.000	0.752	5	1.541	4.792	0.536
Sialia36	7	4.858	6.805	1.706	9	6.015	9.000	1.952	11	6.118	10.267	1.940
Sialia37	5	3.365	5.000	1.386	13	8.511	13.000	2.253	11	8.575	10.824	2.318

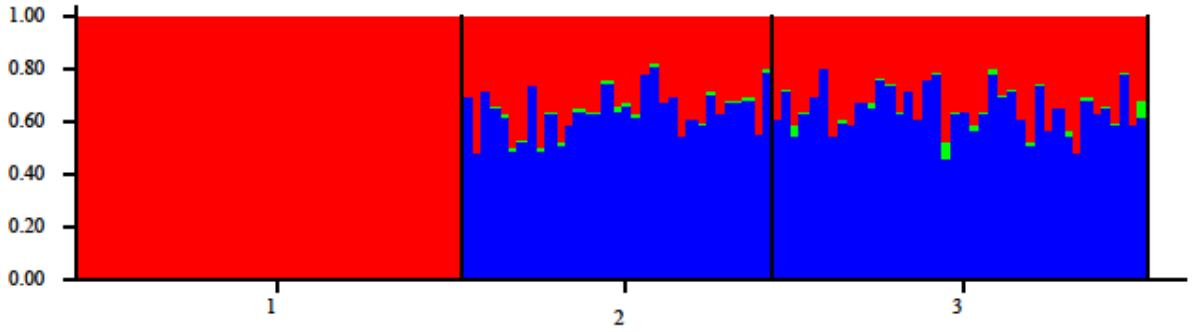
Table S2. Tests of departure from Hardy-Weinberg equilibrium across populations and loci. Values are Weir and Cockerham's F_{IS} values computed in GENEPOP. *significant at p=0.0013

Locus	**Bermuda**	H_O	H_E	p-value	**Cont**	H_O	H_E	p-value	**Coastal**	H_O	H_E	p-value
Sialia2	0.1574	0.415	0.485	0.2418	-0.0535	0.606	0.567	0.9158	0.0331	0.525	0.536	0.2208
Sialia6	-0.1459	0.390	0.337	1.0	0.0171	0.788	0.789	0.5745	0.0321	0.725	0.739	0.3380
Sialia8	0.0278	0.512	0.520	0.5145	0.0171	0.818	0.820	0.5724	0.0446	0.775	0.801	0.1747
Sialia11	0.0531	0.707	0.737	0.6114	-0.0401	0.909	0.861	0.7699	-0.0091	0.850	0.832	0.2087
Sialia15	0.1011	0.195	0.214	0.4597	0.1061	0.455	0.500	0.1953	-.0842	0.450	0.410	0.9250
Sialia18	N/A	0.000	0.000	N/A	-0.0061	0.545	0.534	1.0	0.1684	0.475	0.563	0.2884
Sialia22	0.1727	0.415	0.494	0.2646	0.0789	0.758	0.809	0.0377	-0.0495	0.850	0.800	0.1350
Sialia27	0.0628	0.268	0.283	0.2111	0.1444	0.758	0.870	0.0561	0.1921	0.725	0.884	**0.0000***
Sialia28	-0.0211	0.634	0.614	0.9562	-0.0299	0.848	0.812	0.4364	-0.1425	0.925	0.801	0.4582
Sialia30	0.1646	0.415	0.489	0.3466	0.2381	0.273	0.351	0.3069	0.1450	0.325	0.375	0.1236
Sialia36	-0.1550	0.927	0.794	**0.0003***	0.0373	0.818	0.837	0.7470	0.1429	0.725	0.834	0.0194
Sialia37	0.0753	0.659	0.703	0.3932	-0.0137	0.909	0.883	0.6527	-0.0355	0.925	0.883	0.6840

Table S3. F_{ST}, standardized F'_{ST}, and D_{est}, values across all three sampled regions. P-values are depicted above the diagonal and F_{ST} values are below the diagonal with standardized F'_{ST} values in square brackets and D_{est} values in curly brackets. F'_{ST} represents realized divergence in genetic composition relative to the maximum divergence possible (0 being no divergence and 1 being complete divergence in allele frequencies) and D_{est} depicts actual differentiation also from 0 to 1.

	Bermuda	**Continental**	**Coastal**
Bermuda		0.010	0.010
Continental	0.127; [0.317]; {0.16}		0.460
Coastal	0.128; [0.317]; {0.17}	0.000; [0.000]; {0.00}	

Figure S4. STRUCTURE results for K = 3 populations. Bermuda is section one, Continental is section two, and Coastal is section three.



Chapter 2

Rapid evolution of ornamented plumage and increased sexual dichromatism in an island bird.

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Keywords: Bird, Color, Contemporary Evolution, Dichromatism, Island, Plumage

Data will be archived in supplementary information.

Abstract:

It has long been asserted that island-dwelling birds exhibit less plumage ornamentation relative to their mainland counterparts. Most support for this observation comes from a narrow range of avian taxa and all evidence is derived from subjective human-perceived differences in plumage coloration, making it difficult to trace the mechanisms producing the observed patterns. Using avian perceptual modeling we detail for the first time an exception to the island rule, the eastern bluebird on Bermuda, and show that the increase in ornamented plumage stems from a shift in hue to shorter wavelengths and increased brightness among both males and females, with neither sex exhibiting diminished plumage expression. Sexual dichromatism is enhanced on Bermuda despite the prediction that one sex would change in a way that reduces dichromatism. The Bermudan population of bluebirds was established approximately 400 years ago and these differences, which have convinced some ornithologists to declare the island group a subspecies, have emerged over a relatively short time. Our results provide a compelling avenue for the detailed study of how coloration patterns of birds, or any animal, may change under insular conditions.

Introduction

Islands have contributed a wealth of information on patterns of phenotypic change and the generation of biodiversity, and birds have played a prominent role in this literature (Grant 2001, Doucet et al. 2004, Price 2008, Driskell et al. 2010). An intriguing pattern among island birds is the perceived loss of ornamented plumage, and the reduction of sexual plumage dichromatism, in island versus mainland forms (Grant 1965, Omland 1997, Price 2008). Although often cited as an example of evolution under conditions of insularity, it is not known how comprehensive this pattern is across all avian taxa (Grant 2001), or across island animals in general (e.g., Raia et al. 2010). Furthermore, all existing examples stem from methods that rely on human vision to differentiate plumage color patterns, which we know is (at best) an imperfect and somewhat subjective tool in this context (e.g., Eaton 2005), because it ignores the specific elements that determine coloration as well as the often profound differences in how human and non-human animals (including birds) perceive coloration (Hubbard et al. 2010). Here we describe in detail the unusual pattern whereby an island bird, the eastern bluebird on Bermuda *(Sialia sialis bermudensis)*, has evolved showier plumage and increased sexual dichromatism relative to its mainland source populations, and it has done so within the 400 years since its founding. Our results challenge the existing paradigm regarding plumage coloration in island birds, but they also provide novel insight into the components of vertebrate color that may rapidly change in response to insularity, or limited gene flow.

What animals perceive as color is an integrated sensation of multiple physical stimuli received by the eye. Color can be deconstructed into two components; the chromatic signal with at least three constituents (hue, saturation, and ultra-violet UV

chroma) and the achromatic signal, which is usually described as brightness (Montgomerie 2006). The human eye cannot detect shorter wavelengths in the UV range, however birds and other organisms can. Eaton (2005) argues that the failure to measure UV in past studies of bird plumage dichromatism has biased our understanding of how and when dichromatism arises (or in the case of island birds, diminishes). Although not comprehensively considered in terms of the level of bias it may introduce to past plumage studies, human perception of color integrates hue, saturation and brightness, thus making it impossible to untangle which element of color is changing, and to what extent, when relying on human-derived metrics of color. We understand that some researchers prefer to examine plumage differences as a single unit as a bird likely perceives color, but there is considerable merit in looking at each component individually. One of the principal insights of recent work on bird plumage, which uses objective measures that separate color into its constituent parts, is that the mechanisms behind color production and evolution vary considerably across these color components (Badyaev and Hill 2003, Owens 2006, Stoddard and Prum 2008). To satisfy both views, we test for differences between whole colors and also between the individual components of color.

An oft-cited pattern in the evolution literature is that island bird populations have less ornamented plumage compared to their mainland parental populations (e.g., Bennett and Owens 2002, Price 2008). In some instances the mean plumage color for the island population was shown to have evolved from its 'bright' mainland form towards 'drab' colors on islands (Grant 1965). In other studies the change stems from a reduction in plumage dichromatism between the sexes within the island population thus making the

island forms seem less striking when compared to their mainland counterparts (Peterson 1996, Omland 1997, Price 2008). Most of these studies pre-date the advent of objective quantitative color measurement (Hill and McGraw 2006) leaving us unable to determine what component of color is contributing to perceived plumage differences. Given that the production of each of the color components is different (Hill 2006, Prum 2006), and that each has its own tendency to vary across individuals in a population (Dale 2006), it would be informative to know which components are changing within birds isolated on islands. In addition, it is helpful to know which components of color are the first to change and how fast this may happen after initial isolation, as such information guides our understanding of trait plasticity in the evolutionary trajectory of a species' coloration and eventual speciation (Price 2006, Hubbard et al. 2010).

The Bermuda subspecies of eastern bluebird represents a particularly compelling case study of plumage evolution under conditions of insularization as it seems to defy the commonly accepted rule. The Bermuda bluebird is perceived to be more "brilliant blue" and "purplish azure" than mainland subspecies (Verrill 1901, Bradlee et al. 1931), thereby making it apparently more 'ornamented' than its mainland counterparts (Supplementary Figure 1). These plumage differences are the principal reason why the Bermuda population has been designated as a separate subspecies (Gowaty and Plissner 1998). However, there is very little in the way of quantitative data on this perceived difference, especially in terms of the components of color that have changed to render plumage more brilliantly blue. No one has considered changes in sexual plumage dichromatism in the Bermuda bluebird as compared to its mainland counterparts, or in terms of geographical variation across mainland populations.

The Bermuda bluebird also provides us with the unique opportunity to quantify the magnitude of plumage change over a specified time frame. From genetic analyses, we know that the Bermuda subspecies of eastern bluebird most likely colonized the island in the 1600s (Avery 2012). Although the particular mainland source population is still uncertain, it was likely derived from individuals taken from the nominate eastern bluebird race, which is widespread and genetically panmictic across eastern North America (Avery 2012). This short timeline for plumage evolution to occur is unique in studies of avian feather color; most comparisons are between groups that split from each other >1000 years before present, if not millions of years ago (Rojas-Soto et al. 2010). Thus, we have the unusual opportunity to study plumage evolution in an isolated population going through the very early stages of allopatric speciation.

We know from extensive studies on mainland bluebird populations that structural plumage coloration in this sexually dichromatic species is condition dependent and descriptors of structural color are correlated with measures of fitness, parental care, and mate quality ((Siefferman and Hill 2003, 2005a) c.f. (Peters et al. 2011)). Since the components of plumage coloration (hue, saturation, brightness and UV chroma) are under varying levels of genetic versus environmental control, our documenting which components have changed within Bermuda bluebirds relative to the mainland populations provides insight into the mechanism of plumage evolution. Hue signals genetic quality of males in mainland populations of eastern bluebirds (Johnsen et al. 2003, Siefferman and Hill 2003), thus we may expect this color component to be more susceptible to the effects of genetic drift and founder events. By chance, individuals that founded the Bermuda population, or that survived the early years of colonization of the island, could have had a

suite of genes that coded for a hue and saturation that looks ‘bluer’ to the human eye. It is also possible that heightened sexual selection pressures on the males on the island cause each of these color components to produce bluer plumage since they signal mate quality in mainland bluebird populations (Siefferman and Hill 2005b, c). This mechanism of plumage evolution may be particularly likely given the limited nesting opportunities and the relatively high densities of bluebirds on the island as compared to the mainland (Wingate pers. comm.) (Prentiss 1896), a situation typical of island vertebrates in general (Bourne 1957, Wright 1980, Whittaker and Fernandez-Palacios 2007). In contrast, brightness and UV chroma are condition-dependent in mainland bluebirds (Siefferman and Hill 2005c), and therefore are quite variable between individuals. Thus, these more plastic components of plumage color may respond quickly to the local ecological conditions on Bermuda (Prum 2006). If these conditions are sufficiently different on Bermuda than on the mainland, this mechanism will lead to a human-perceived bluer coloration of the individuals that reside there.

We also compared the degree of sexual plumage dichromatism between the mainland and Bermuda populations. We have two lines of evidence that predict bluebirds should be less dichromatic on Bermuda. First, Bermuda bluebirds are non-migratory, which seems to confer an increase in the coloration of female plumage while having little effect on male plumage (Peterson 1996, Fitzpatrick 1998). We include both a migratory and non-migratory mainland population in our study so that we can gauge the extent to which Bermuda bluebirds conform to the dichromatism patterns typical of non-migratory bluebird populations. Second, island populations show a decrease in dichromatism through either a decrease in male plumage coloration or an increase in female coloration

(see above) (Omland 1997, Price 2008). Thus, we expect male and female dichromatism to be lessened in Bermuda, although we have no a priori expectations for which of the two sexes will converge with the other, or if they both will essentially meet in the middle. Given that we expect Bermuda bluebirds to be 'bluer', it will also be of interest to document whether only one sex is contributing to this perception, or if both are contributing.

Materials and Methods

We collected three body feathers from the rump of adult bluebirds captured on Bermuda and the coastal (non-migratory) and continental (migratory) regions of North America (Fig. 1) during June and July of 2007 and 2008. We chose to use feathers from the rump patch to be consistent with previous studies on bluebirds (Shawkey et al. 2005, Siefferman and Hill 2005a). Also we found that rump feather coloration is highly correlated with the coloration of feathers collected from other body patches (i.e. head and back, data available on request). Finally, rump feathers showed high variability in color metrics (data available on request) and high variability is often assumed to indicate the conveyance of more information to receivers (Dale 2006).

To approximate their natural arrangement on the bird, we stacked the feathers collected from each individual on a black velvet background that had zero reflectance (Siefferman and Hill 2003, Shawkey et al. 2005). We used an Ocean Optics USB4000 spectrometer and Ocean Optics PX-2 pulsed xenon light source to quantify plumage color. All measurements of reflectance were calculated relative to a diffuse white standard (Ocean Optics WS-1) using SpectraSuite (2006). Each measurement of a

plumage patch is an average of 10 scans computed within the spectrasuite software during data collection. We inserted the measurement probe into a matte black plastic sleeve that prevented ambient light from entering the read fibers thus creating a measurement distance of 5mm. We made five repeated measurements of each feather set by picking up the probe and placing it back down within the plumage. We calculated measurement error (ME) (Bailey and Byrnes 1990) and found it to be consistent with other published results from structural coloration (Budden and Dickinson 2009). Male and female hue was most repeatable (9.9% and 7.0% ME respectively) and UV had moderate repeatability (37.4 and 32.7 respectively). Both male and female saturation (65.9% and 45.8%) and brightness (51.85% and 56.5%) exhibited higher ME. We then averaged the five repeated measurements to generate a single reflectance curve for each individual. The range of wavelengths captured by this process included 300 to 700nm and thus includes all parts of the avian visible spectrum.

Birds are tetrachromats and they perceive color through the use of four cone photoreceptor classes with differing sensitivities to incoming light. Most bird species are capable of seeing a broader range of incoming light that encompasses portions of the UV spectrum. They do this through the use of two short wavelength sensitive (SWS1 and SWS2) photoreceptors (mammals have SWS2 only) where one has peak sensitivity 355 – 445 nm and the second, shared with primates, has peak sensitivity 400 – 470 nm (Hunt et al. 2009). Thus, to obtain measures of hue, saturation, and brightness free from human perception and relevant to avian visual systems, we used the program TETRACOLORSPACE (Stoddard and Prum 2008). For the reflectance curve from each individual, TETRACOLORSPACE calculates the photon catch for all color receptors present

in the avian visual system, and plots the position of that color in a tetrahedral color space using the following formula,

$$Q_I = \int_{300}^{700} R(\lambda) C_r(\lambda) d\lambda$$

where Q_I is the idealized stimulus for each of four avian cone types sensitive to ultraviolet wavelength, short wavelength, medium wavelength, and long wavelength light integrated across all wavelengths (λ) between 300nm and 700nm. $R(\lambda)$ is the reflectance spectrum of the plumage patch. $C(\lambda)$ is the spectral sensitivity function for each of the four avian cone types, and $d(\lambda)$ is a constant. We did not use an irradiance spectrum, and we calculated photon catches using average spectral sensitivity curves for a theoretical model ultraviolet sensitive bird. The stimulation values from all cones are used to calculate the three-dimensional coordinates (X, Y, Z) of each color point in tetrahedral space following Stoddard (2008).

The Cartesian coordinates for a color in the tetrahedron are converted to spherical coordinates, θ, r and ϕ that represent actual components of the color signal. The horizontal angular displacement from the positive x-axis around the origin is θ. This value lies purely within the x-y plane and is equivalent to the hues visible to humans. The variable r represents saturation, the purity of a hue and a measure of how much white exists in that hue. The angular measurement ϕ describes the UV contribution to hue, but is not equivalent to percent UV because it describes a direction, and thus must include the value of r, or the distance of a color from the origin to capture how much UV reflectance is present. A constant angle ϕ could represent different amounts of UV reflectance as r varies. Because ϕ by itself does not represent percent UV and could be misleading, we

decided not to include it in the analysis and instead obtained Z from the ultraviolet cone stimulus values. The Z-axis then represents percent UV. These color variables are processed independently from brightness, which can affect how a color is perceived. We thus used TETRACOLORSPACE to calculate normalized brilliance (total reflectance / N * 100) of each color patch as our measure of plumage brightness (Stoddard and Prum 2008).

All statistical analyses were performed using the program R version 2.14.1 (R Development Core Team 2011). We have archived the data used in all analyses in online supplementary information. We first used the Cartesian coordinates of each color point to compare overall differences in color between island and mainland individuals, and to calculate measures of dichromatism between the sexes. We tested for separation between sexes and regions with a PERMANOVA (Anderson 2001). In our PERMANOVA we used 1,000 permutations to test for overall differences in coloration between regions (mainland migratory, mainland non-migratory, Bermuda) and between sexes using the Euclidean distance measure in the package VEGAN (Oksanen et al. 2011). The Cartesian coordinates X, Y, and Z were the dependent multivariate response variables and region (three levels) and sex (two levels) were the independent variables. We used a PERMANOVA because each Cartesian response variable was non-normal and correlated with one another, and we wanted to test for separation between groups of color points. This procedure is ideal for data that do not follow normal distributions and exhibit colinearity (Anderson 2001). This test allowed us to make inferences about sexual dichromatism and whole plumage colors, setting the stage for tests of individual color descriptors (e.g., hue, saturation, UV, and brightness). Our initial model included two

factors, region (three levels), and sex (two levels), as well as the interaction between region and sex. We followed this global model with a PERMANOVA for each sex using region as the independent variable to test for sex-specific differences across regions.

Given a statistically significant PERMANOVA for the overall segregation of color points, we conducted univariate tests of differences between regions in the color components hue (θ), saturation (*r*), UV, and brightness. We used general linear models (GLM) because the data were unbalanced with unequal numbers of observations across regions. Males and females were significantly different in overall coloration (see below), therefore we ran the GLMs for each sex independently and included region as the explanatory variable. All individual color variables with the exception of male saturation followed a normal distribution. We were unable to satisfactorily transform male saturation and therefore used the non-parametric test described above to look for differences across regions. The results were essentially the same as a univariate GLM so we reported the test statistics from a GLM to match output for other variables.

To quantify differences in sexual dichromatism within a region, we built a matrix of all possible Euclidean distances between male and female color points in our dataset using the three Cartesian coordinates. To visualize region-specific mean dichromatism, we used a bootstrap procedure to sample inter-sexual distances from this matrix for each region, repeating this sampling routine 10,000 times. We then calculated the mean dichromatism value from each of the 10,000 iterations. We calculated 95% confidence intervals on the bootstrapped means to look for overlap in levels of sexual dichromatism between regions.

Results

We found evidence for overall differences in blue coloration across geographical regions (Fig 2) (region: $F_{2,109} = 10.34$, $P < 0.001$; sex: $F_{1,109} = 278.34$, $P = 0.001$; region*sex: $F_{2,109} = 2.32$, $P = 0.083$). Rump coloration differed between Bermuda and both mainland populations, but not between mainland populations. Male and female PERMANOVAs showed a similar result when all three regions were included ($F_{2,54} = 6.47$ and $F_{2,55} = 7.34$, $P = 0.001$ and 0.001 respectively). This region effect disappeared when we removed Bermuda and tested the mainland male and female groups across mainland regions only ($F_{1,19} = 0.22$ and $F_{1,30} = 0.90$, $P = 0.816$ and 0.394).

Males and females followed the same pattern for each individual color variable as the PERMANOVA above (Fig 3, A and B). Continental and coastal populations never exhibited differences in any color variable that set them apart from each other. The strongest differences between Bermuda and the mainland regions were seen for hue and brightness. Bermuda bluebirds were consistently more violet in hue than the mainland individuals (more negative θ), and mean brightness for Bermuda bluebirds was considerably higher for both sexes as compared to mainland individuals, although the range of brightness values was broad in all regions. Saturation and UV were not different across geographical regions with the exception of female UV, which was significantly lower in the island population. Within univariate tests that showed a significant difference in a color variable, Bermudan individuals possessed the most extreme values in both sexes. One thing to note from the boxplots for male color (Fig 3A) is that the variability of hue and brightness is greater for Bermudan individuals than the variability in the mainland population. Bermuda individuals at times exhibit values similar to

individuals from the mainland but they also express plumage colors that we perceive as more vivid and indigo than what is seen among the mainland individuals.

With respect to plumage dichromatism, as predicted, we found sexual dichromatism was reduced in the non-migratory mainland population relative to migratory mainland populations. Contrary to our expectations, however, Bermuda exhibited higher sexual dichromatism than the non-migratory mainland population (Fig 4), although the degree of dichromatism in Bermuda is indistinguishable from that observed in the migratory mainland population (95% confidence intervals; Bermuda 0.054 – 0.059, migratory 0.056 – 0.067, non-migratory 0.029 – 0.033) . Sexual dichromatism seems to be higher in the Bermuda population as compared to the non-migratory mainland population as a result of both sexes increasing in trait values, with males increasing slightly more than females (Fig. 3A, B).

Discussion

We detail for the first time, with quantitative data, an exception to the pattern of drab island plumage using the eastern bluebird on Bermuda, and show that the increase in ornamented plumage of Bermuda bluebirds stems from a change in hue and an increase in brightness among both male and female individuals, with neither sex exhibiting diminished plumage expression. In other words, sexual dichromatism is enhanced on Bermuda despite the prediction that one or the other sex would change in a way that would instead reduce dichromatism. Because the Bermudan population of bluebirds was established around 400 years ago (Avery 2012), these differences, which are large enough to have convinced some ornithologist to declare the island group a subspecies,

have emerged over a very short time span. Our results provide a compelling avenue forward for the detailed study of how coloration patterns of birds, or any animal, may change under conditions of insularity.

Bermudan male and female bluebirds possess feathers that are more purplish in hue and brighter than mainland populations, however these same individuals show no difference in color saturation or UV chroma with the exception of female UV chroma. These contrasting shifts in color components provide insight into the mechanisms driving the increased ornamentation of Bermuda bluebird plumage. Within mainland eastern bluebirds brightness is most often associated with the physical condition of individual birds (bright feathers equate to good physical condition), which almost certainly reflects that individual's ability to secure and defend prime habitat (Hamilton and Zuk 1982, Siefferman and Hill 2005a). Accordingly, brightness tends to be quite variable between individuals in mainland populations and is often the putative subject of sexual selection (Siefferman and Hill 2005b). Saturation is also highly variable between individuals on the mainland in part because it too is tied to the physical condition of individuals, but saturation did not differ between Bermudan and mainland individuals. If plumage differences between island and mainland individuals were due solely to ecological factors, we should expect to see both brightness and saturation vary together. The fact that they do not suggests that (1) if the ecological conditions are quite different on Bermuda relative to eastern North America, the developmental pathways that influence saturation and brightness respond very differently to ecological conditions, (2) sexual selection is operating differently for plumage saturation versus brightness, or (3)

ecological conditions are nearly the same across regions and instead brightness has a stronger genetic component to its expression than previously suspected.

The range of variation in hue values across individuals in Bermuda is unexpected since hue tends to be remarkably similar across individuals in mainland populations and has been shown to be under genetic control (Johnsen et al. 2003). It is possible that resources may be limited on Bermuda and fitter individuals with greater expression of hue are able to control resources more efficiently, leaving less fit birds with fewer resources during feather development and thus a different hue value. We know from previous research that neutral genetic variation was significantly lower within the Bermuda population as compared to the mainland populations (sampled at the same locations as plumage color) (Avery 2012). From this we determined that the Bermuda bluebird population has undergone a genetic bottleneck likely around the time of their founding (1600s). Thus, Bermuda bluebirds are achieving variation in expression of plumage hue that well exceeds mainland levels despite a significant loss of (neutral) genetic variation.

The absence of changes in male UV expression is surprising based on previously published work on mainland eastern bluebirds (Siefferman and Hill 2003, 2005a). It is always possible that our sample sizes were too low to be able to detect more subtle shifts in these color components, especially if environmental conditions are highly variable with these color components closely tracking these conditions. However, a change in UV expression should accompany a change in hue because the shift in hue should alter the shape of the reflectance curve, causing differential stimulation of the UV sensitive cones. UV contributes substantially to the avian color experience so it seems unlikely that

plumage color would vary only in the range humans can detect and not across the avian visible spectrum.

The pattern of elevated sexual dichromatism in the sedentary Bermuda population is even more unexpected. The average separation in plumage coloration between males and females on Bermuda is not different than that for migratory individuals on the mainland. This is true despite the increased hue and brightness of female bluebirds on Bermuda, which is expected in sedentary populations (Peterson 1996). However this increase in female coloration was offset by a slightly more pronounced increase in male plumage ornamentation for which there is no known precedent in the literature. It is possible that females have been able to express greater ornamentation because they are released from the resource demands associated with seasonal migration. Meanwhile, sexual selection on males may have increased on the island through increases in extra-pair paternity, despite the expectation that sexual selection is relaxed on islands (Griffith 2000, Price 2008). If males frequently keep the same trait value in sedentary populations while females increase expression, then the additional resources gained through loss of migratory behavior is not a likely explanation for increased male traits on Bermuda.

To our knowledge, our study is the first comparison of divergence in plumage characteristics where the time of divergence of the island population from the mainland is known with some accuracy, and certainly the first to explore the possibility that such changes can occur over contemporary time frames (<400 years). Thus our results strongly suggest that plumage coloration is quite evolutionarily labile, even when considering color components that are typically thought to be under stabilizing selection within mainland populations (e.g. hue (Omland and Lanyon 2000)). Our results are all the

more striking given the lack of differentiation in bluebird plumage across a broad region on the mainland. Traits on the mainland are conserved across populations despite strongly divergent environmental and life history traits (e.g., seasonal versus non-seasonal climates and migratory versus non-migratory behavior) and known variation at the level of the individual (Siefferman and Hill 2005c). These observations suggest to us that Bermuda bluebirds are experiencing strong selection rather than drift, because drift would not act on both hue and brightness in tandem (one under genetic control and the other environmental). Furthermore, drift is more likely to lead to a constraint in phenotypic expressions such as plumage coloration due to a large reduction in genetic variation whereas on Bermuda the range of color traits is the same, if not expanded, as compared to mainland populations. Whether this selection is driven by ecological factors particular to Bermuda is beyond the scope of this study. However, there is evidence that ecological factors can influence coloration in the face of ongoing gene flow (Rojas-Soto et al. 2010). This possibility stresses the importance of genetic isolation in the patterns we see on Bermuda. Isolation was likely needed to overcome the inherent resilience of these color traits thereby opening a window and creating an opportunity for incipient speciation.

There are two other factors that should be considered as potential explanations of plumage differences between mainland and Bermuda bluebirds. First, humans may have introduced bluebirds to Bermuda (Avery 2012) and may have selected founding individuals that were particularly ‘appealing’. Therefore there may have been an initial element of artificial selection for more colorful individuals. Second, the initial founding event may have increased the rate of adaptation by increasing additive variation. This

could occur through shifts in the frequency of loci with non-additive gene interactions (Dlugosch and Parker 2008). However, population bottlenecks can reduce overall genetic variation and make it harder for traits to reach extreme phenotypes. Because we see extreme values of color components in the Bermuda population that are not attained on the mainland we believe selection rather than human influence is playing a strong role in observed differences.

Research on the evolution of coloration in vertebrates has seen remarkable advances over the past decade. This advancement has come via our increased understanding of the genetic components of color production (Hubbard et al. 2010), and our ability to measure color as it is perceived by the individual(s) responding to the information color provides (Hill and McGraw 2006). These advances open a variety of avenues for deeper exploration of how coloration responds to a diversity of environmental and social conditions, and opens investigative doors into patterns that have long held our attention. Studies that use objective methods to compare attributes of color across geographic ranges are extremely rare, especially amongst vertebrates, and are non-existent for cases where island forms are compared to mainland source populations. Our results suggest that coloration patterns may be quick to respond to a change in environmental and social conditions, especially those that accompany insularization, even in cases where the colors involved have complex control mechanisms (i.e. are not simple mutations that result in binary color patterns). Our results for the Bermuda bluebird lead to a variety of testable hypothesis as to how these individuals become ‘bluer’. However on a broader scale, our results shed light on possible evolutionary mechanisms behind some fantastic examples of adaptive radiation in island forms, such as Hawaiian

honeycreepers, where coloration seems to have been elaborated through time and has contributed to the formation of species.

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Table 1. Univariate GLM results for each color variable as measured in males and females. We used region (Bermuda, migratory mainland, and non-migratory mainland) as the independent variable in all tests. Significant tests describe which components of blue coloration contribute to overall differences between populations

	Male			**Female**		
Trait	DF	F	P	DF	F	P
Hue	2, 54	15.73	0.000	2, 55	31.32	0.000
Saturation	2, 54	0.97	0.385	2, 55	0.35	0.704
UV	2, 54	2.39	0.102	2, 55	9.14	0.000
Brightness	2, 54	16.40	0.000	2, 55	7.05	0.002

Figure 1. Map of North American and Bermudan sampling localities. Samples were aggregated so that the migratory group comprised all Minnesota and Iowa (10 males, 20 females) individuals and the non-migratory group contained New Jersey and North Carolina individuals (11 males, 12 females). Shading represents the range of nominate subspecies *S. s. sialis* in the United States.

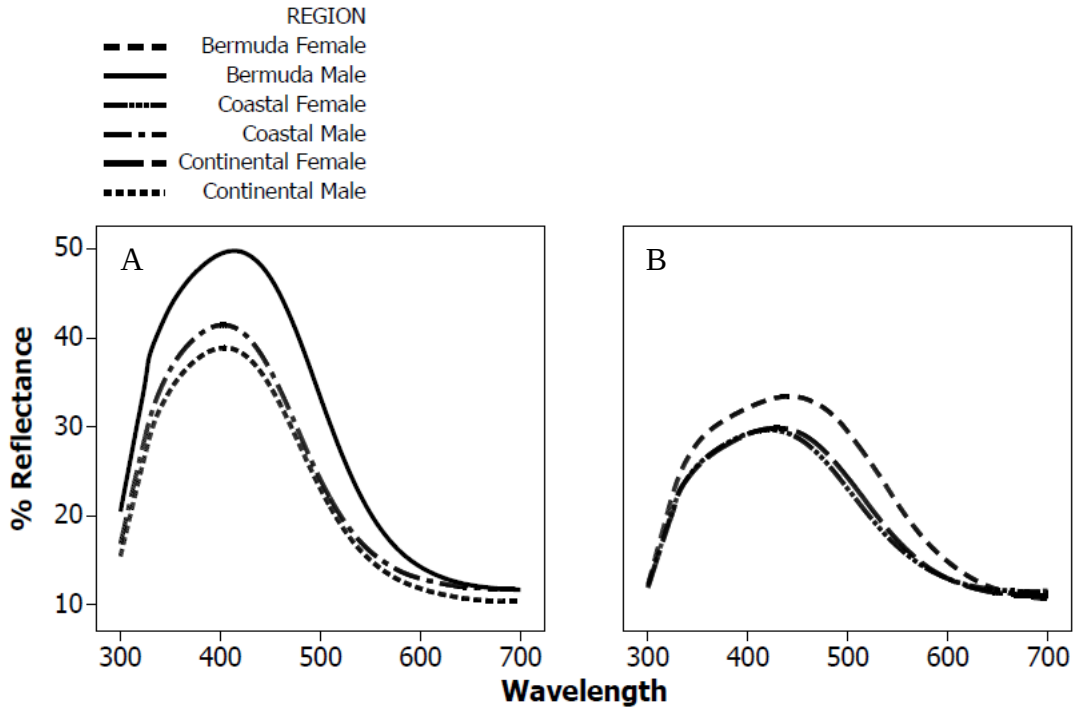

Figure 2. Average reflectance spectra for males (A) and females (B) in each region. Percent reflected light is along the y-axis and represents how much light is reflected at each wavelength (x-axis). Bermuda males and females show greater reflectance than both mainland regions.

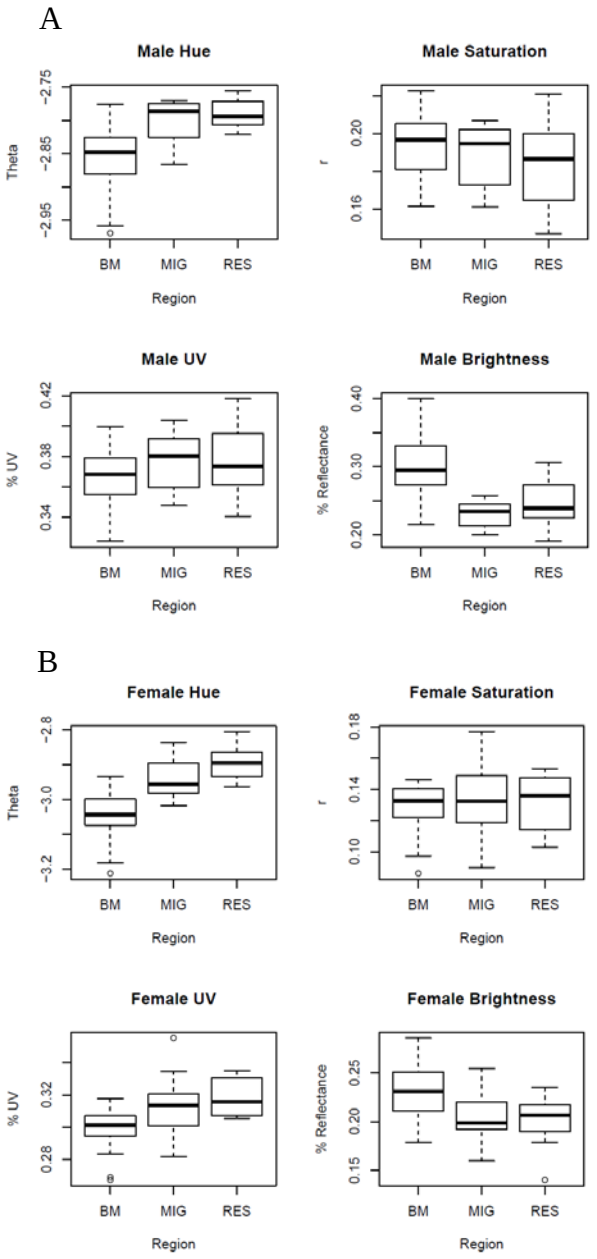

Figure 3. Individual descriptors of eastern bluebird plumage color across all males (A) and females (B) in Bermuda, the migratory mainland group, and the non-migratory mainland group. Hue is the color humans would perceive as it does not include UV light. Saturation is a measure of spectral purity. UV is percent reflectance in the ultraviolet portion of the spectrum. Brightness is a measure of total plumage reflectance.

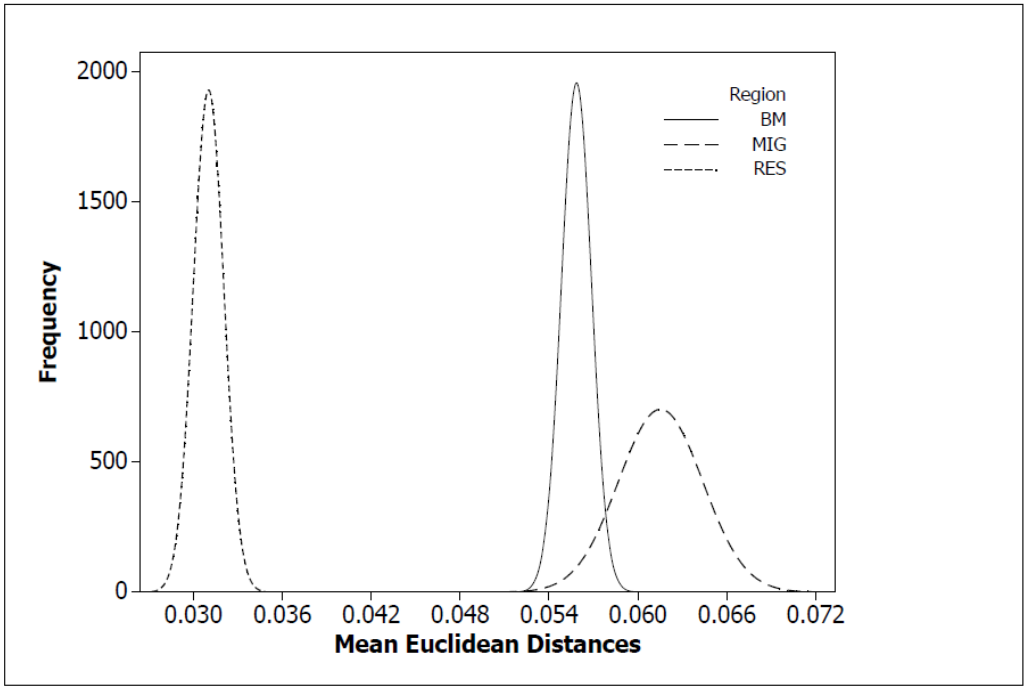

Figure 4. Probability density curves of mean Euclidean distance values between males and females bootstrapped 10,000 times for each region.

CHAPTER 3

Differential contribution of color signals to avian plumage diversity

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Abstract

Bird plumage color is incredibly diverse, yet the mechanisms that contribute to the maintenance and generation of this diversity are poorly understood. This difficulty stems from our limited understanding of intraspecific variation in the components of a color signal. Color signals are complex because they consist of at least four components and each of these components varies with respect to different factors. Since plumage color influences the maintenance and production of species limits, an understanding of which color components are contributing to differences in plumage between avian groups is critical to our understanding of the generation of taxonomic diversity. We employ avian perceptual modeling to investigate the variability of blue plumage across subspecies of eastern bluebirds (*Sialia sialis*). Our results suggest that differences in perceived plumage coloration are driven much more by the components of color that reflect the ecological conditions of a geographical location rather than components that are genetically determined.

Keywords: Birds, Color, Chroma, Evolution, Hue

Introduction

Bird plumage color appears to exhibit a limitless range of possibilities. Birds are the most colorful land vertebrates [1], and rank highly among all taxa in the variety of colors they display [2]. There are myriad mechanisms that can produce such diversity in bird plumage. However, teasing apart these mechanisms is difficult in part because we have little understanding of how the components of color vary between taxonomic groups. What any animal perceives as color represents the combined influence of the way that light is reflected off an object and the visual perception and psychological integration of these light waves by the animal itself [3]. Thus, 'color' is not a single trait that is easily categorized, but instead is a complex signal that can be deconstructed into specific components. Moreover, these components cannot be adequately understood in terms of their contribution to diversity without reference to how they are perceived [3]. Since plumage color influences the maintenance and production of species limits [4,5], an understanding of which color components are contributing to differences in plumage between avian groups is critical to our understanding of the generation of taxonomic diversity [2]. We employ avian perceptual modeling to investigate the variability of blue plumage across subspecies of eastern bluebirds (*Sialia sialis*). Our results suggest that differences in perceived plumage coloration are driven much more by the components of color that reflect the ecological conditions of a geographical location rather than components that are genetically determined.

In the past when ornithologists described differences in plumage between species or subspecies, they used color metrics that were necessarily derivatives of how humans perceive color. They did not have a choice in this decision since technological advances

in measuring light, and in modeling color perception, have become available only over the last decades [6]. Based on these recent technological advances, today's biologists deconstruct color expression into two parts; the chromatic and achromatic signals. The chromatic signal itself consists of three components; hue, chroma and ultra-violet. Hue is the technical term for color, chroma is a measure of a hue's purity in the human-visible spectrum (400 – 700nm), and ultra-violet reflectance (percent UV) is a measure of the light reflected between 300 and 400nm; a region detectable to birds but not humans. The achromatic signal, called brightness, is psychologically processed by vertebrates independent of the chromatic signal, although it can greatly influence how these other components are perceived [3].

One of the principle insights from recent work on bird plumage is that the mechanisms behind color production and evolution vary considerably across these color components [7-9]. Thus, we cannot adequately interpret variation in plumage color across taxa until we improve upon our understanding of intraspecific variation in color's components [10]. There have been several recent efforts to distinguish true plumage color differences between closely related avian taxa using modern methods of quantifying color [e.g., 4,11]. However, this research aimed at improving our ability to classify taxa based on plumage color; they did not explicitly address how the color components were contributing to plumage diversity nor did they consider plumage differences as perceived by birds themselves. Thus, we are left with little understanding of which color components are involved in observed differences in plumage color between taxonomic groups.

In contrast to the above research on macro-scale plumage color differences, there is a considerable body of work detailing the correlation between plumage color components and measures of individual quality [12-15]. Several species have become 'models' in this regard (e.g., house finches *Carpodacus mexicanus* and pied flycatcher *Ficedula hypoleuca*), including the eastern bluebird. The blue plumage of bluebirds is produced by feather microstructure that is capable of coherent light scattering [16]. Male and female bluebirds show differing magnitudes of blue in their head and rump feathers [16]. Blue feather color is also known to be condition and age dependent in male bluebirds. These individual differences in male and female blue plumage are correlated with measures of fitness, parental care, and mate quality [12,17] c.f. [18]. For example, female bluebirds choose males for mating based on the brightness and percent UV of a male's blue feathers as these are honest signals of his quality and parental care [17].

Despite this detailed understanding of how blue feather color is produced in bluebirds, and its importance in mate choice, we know very little about how these components of blue vary across higher taxonomic units [c.f. see 19 for differences in feather microstructure across bluebird species]. The situation for bluebirds is not unusual. Variation in color signal components has rarely been scaled up from the individual to broad geographical scales to show how taxa collectively express plumage color variation [9,20-22]. As a result, we do not know if individual color component variability is indicative of macro-scale differences between taxa. For instance, does brightness contribute to variability among populations or subspecies in the same way it differs between individuals? In essence, macro-scale patterns in plumage differences have been regularly described, however we have very little information on the route taken to reach

such differences. This is analogous to a mariner seeing a protected harbor but not having an appreciation for the underlying channels that would enable his safe arrival at that destination. To bridge this gap between scales, we have to chart the color channels between individual and group differences.

The genus *Sialis* is part of a basal lineage of true thrushes consisting of three distinct species occupying North and Central America [23]. These species likely diverged from one another relatively recently in the Miocene [23]. The eastern bluebird is by far the most widespread of the *Sialis*, and exhibits substantial geographical variation in plumage color and morphology. Gowaty and Plissner [24] recognize eight subspecies of eastern bluebirds, which range across eastern North America, Bermuda, Mexico (including southeastern Arizona), Belize, Guatemala, El Salvador Honduras, and Nicaragua (See Figure 1). There has been no effort to substantiate the status of these subspecies using molecular systematics, and thus classification is based in large part on human-perceived blue plumage differences.

From the detailed work on individual differences in blue plumage described above, we know the components of blue coloration are under varying levels of genetic versus environmental control. Since hue signals genetic quality of males [14,17], we expect this color to have a high heritability and thus be less variable across bluebird subspecies. In contrast, brightness, chroma and percent UV are condition dependent [25], and therefore are quite variable between individuals. These more evolutionarily plastic components of bluebird plumage color may respond quickly to local ecological conditions and thus we expect them to vary considerably across subspecies [26]. Given these expectations, we address the following three questions; 1) to what extent do the

components of blue plumage color in eastern bluebird co-vary with each other, 2) which components of blue plumage color differ across eastern bluebird subspecies, and 3) do these four color components contribute equally to any differences between groups or do some play a disproportionate role. We expect more isolated subspecies (increasing geographic distance) to exhibit greater plumage divergence.

Methods

We measured 359 museum specimens from seven of the eight eastern bluebird subspecies recognized by Gowaty and Plissner (1998). These specimens are housed at the American Museum of Natural History, Natural History Museum at Tring, Harvard Museum of Comparative Zoology, United States National Museum of Natural History, and the Chicago Field Museum. Of these specimens, 220 were male and 139 were female. We restricted specimen collection date between the years 1871 and 1941. Bluebirds molt once annually after their breeding season ends in August [24], therefore we chose to restrict our analysis to specimens collected between February and July. We used feathers from the rump patch in order to be consistent with previous studies on eastern bluebirds [12,16]. We also found that rump feather coloration is highly correlated with the coloration of feathers collected from other body patches (i.e. head and back, data not shown). Finally, rump feathers showed high variability in color components (data not shown) and high variability is often assumed to indicate the conveyance of more information to receivers [10].

We used an Ocean Optics USB4000 spectrometer and Ocean Optics PX-2 pulsed xenon light source to quantify plumage color. All measurements of reflectance were

calculated relative to a diffuse white standard (Ocean Optics WS-1) using SpectraSuite (2006). Each measurement of a plumage patch is an average of 10 scans computed within the spectrasuite software during data collection. Integration time was 100ms with a strobe delay of 100ms and boxcar averaging set to zero. We inserted the measurement probe into a matte black plastic sleeve that prevented ambient light from entering the read fibers thus creating a measurement distance of 5mm. We made five repeated measurements of each individual by picking up the probe and placing it back down on the plumage. We then averaged the five repeated measurements to generate a single reflectance curve for each individual. The range of wavelengths captured by this process included 300 to 700nm and thus includes all parts of the avian visible spectrum.

Birds are tetrachromats and they perceive color through the use of four cone photoreceptor classes with differing sensitivities to incoming light. Most bird species are capable of seeing a broader range of incoming light than humans that encompasses portions of the UV spectrum. They do this through the use of two short wavelength sensitive (SWS1 and SWS2) photoreceptors (mammals have SWS2 only) where one has peak sensitivity 355 – 445 nm and the second, shared with primates, has peak sensitivity 400 – 470 nm [27]. To obtain measures of hue, chroma, percent UV and brightness free from human perception and relevant to avian visual systems, we used the program TetraColorSpace [8]. For the reflectance curve from each individual, TetraColorSpace calculates the photon catch for all color receptors present in the avian visual system, and plots the position of that color in a tetrahedral color space using the following formula,

$$Q_I = \int_{300}^{700} R(\lambda) C_r(\lambda) d\lambda$$

where Q_I is the idealized stimulus for each of four avian cone types sensitive to ultraviolet wavelength, short wavelength, medium wavelength, and long wavelength light integrated across all wavelengths (λ) between 300nm and 700nm. R(λ) is the reflectance spectrum of the plumage patch. C(λ) is the spectral sensitivity function for each of the four avian cone types, and d(λ) is a constant. We did not use an irradiance spectrum, and we calculated photon catches using average spectral sensitivity curves for a model ultraviolet sensitive bird. The stimulation values from all cones are used to calculate the three-dimensional coordinates (X, Y, Z) of each color point in tetrahedral space following Stoddard [8]. Each apex of the tetrahedron represents one of the four avian cone types.

To describe plumage color in a way that relates to how humans perceive color, the arbitrary Cartesian coordinates for a color in the tetrahedron are converted to spherical coordinates, θ, r and ϕ that represent actual components of the color signal. The horizontal angular displacement from the positive x-axis around the origin is θ. This describes color as a human (with only three cones) would perceive it; purely within the x-y plane and equivalent to the hues visible to humans. The variable r represents chroma, the purity of a hue and a measure of how much white exists in that hue. The angular measurement ϕ describes the UV contribution to hue, but is not equivalent to percent UV because it describes a direction, and thus must include the value of r, or the distance of a color from the origin to capture how much UV reflectance is present. A constant angle ϕ could represent different amounts of UV reflectance as r varies. Because ϕ by itself does not represent percent UV and could be misleading, we decided not to include it in the analysis and instead obtained Z from the ultraviolet cone stimulus values. The Z-axis then represents percent UV. These color variables are processed independently from

brightness, which can affect how a color is perceived. We thus used TetraColorSpace to calculate normalized brilliance (total reflectance / N * 100) of each color patch as our measure of plumage brightness [8].

We evaluated measurement error (ME) for each color component following the methods of Bryne and Bailey (1990).

$$\%\, ME = 100\%\, (\, s^2_{within} \div (s^2_{within} + s^2_{among})$$

We used the five repeat measures of each color component as our response variable in ANOVA and the sample ID was our independent variable. Although we took all precautions to maintain a measuring angle of 90°, we expect relatively high measurement error since blue coloration is determined by detailed feather microstructures. Thus, the light captured by the spectrophotometer will be highly influenced by the exact angle at which feathers are approached by the measurement probe. High measurement error makes our statistical tests conservative in that they must detect differences within data that is relatively 'noisy'.

We evaluated the extent of correlation between the components of blue feather color using Pearson's product moment scores. Each variable was compared against the other three across all subspecies combined and statistical significance was measured using a two-tailed test with alpha set at 0.05.

Specimen age has been shown to have subtle effects on plumage color [20,28]. We determined through regression analysis that specimen age (year collected) and collection month each had small but significant effects on plumage color. Birds in fresher plumage (earlier months) exhibited greater chroma and brightness, and older specimens

had slightly less UV reflectance. While these effects were small, we chose to control for their influence on our analyses by including them as covariates in analyses below.

To test for the presence of detectable differences in plumage coloration between subspecies we first used the Cartesian coordinates of each color point in a PERMANOVA [29]. We used PERMANOVA because of colinearity between variables (see Results) and our inability to transform some non-normal variables (chroma). In our PERMANOVA we used 1,000 permutations to test for overall differences in plumage coloration between subspecies with collection year and month as covariates using the Euclidean distance measure in the package VEGAN [30]. The Cartesian coordinates X, Y, and Z were the dependent multivariate response variables and subspecies (seven levels) was the independent variable.

We followed each significant PERMANOVA with univariate tests for differences in subspecies in the individual color components [11,20]. Subspecies was again the independent variable and collection year and month were covariates. All individual plumage color components with the exception of male chroma followed a normal distribution. We were unable to satisfactorily transform male chroma and therefore used the non-parametric test described above to look for differences across subspecies. The results were essentially the same as a univariate ANCOVA on male chroma so we reported the test statistics from ANCOVA. After each significant univariate test we conducted Tukey's post-hoc pairwise comparisons to determine the manner in which each component differed between subspecies.

We used discriminant analysis (DA) to determine if color components contributed equally to any observed differences across subspecies [6,31]. For each of the four color

components, DA calculates the unique contribution of each to the discrimination of subspecies. Those components with larger discriminant function coefficients perform better at differentiating between groups. In other words, those components that discriminate well are also those that differ the most between subspecies. In both male and female DA the first two components explained > 90 % or more of the total variation. All statistical analyses were performed using the program R version 2.14.1 [32].

Results

Measurement error was highest in measures of male and female chroma (44.7 and 49.3 % ME) and brightness (39.6 and 34.4 % ME). Measures of male hue were more accurate (9.9% ME) whereas female hue (30.7% ME) and male and female percent UV (29.2% and 27.1% respectively) exhibited moderate error relative to the other components of plumage color. These measures of % ME are similar to other reported values for structural blue coloration [33,34].

The results of our correlation matrix (Table 1) between color variables show high correlation between hue and percent UV, and between chroma and percent UV. All other components were significantly correlated, albeit only moderately. It deserves mention that brightness, processed independently from color, is not strongly associated with any of the other three components. Hue and chroma are both strongly associated with percent UV but not with one another.

Both male and female PERMANOVAs showed a significant effect of subspecies on plumage coloration (male: $R^2 = 0.12$, $F6_{,212} = 4.85$, $P = 0.001$ and female: $R^2 = 0.20$, $F_{6,131} = 5.39$, $P = 0.001$). Once we determined there was separation of bluebird plumage

in avian visual space on the basis of subspecies (Figure 2), we proceeded to our univariate analyses of color components. Our ANCOVA results show a significant effect of subspecies on all plumage color components for both sexes (Table 2). These effects remain strong after controlling for the influence of collection date and month upon coloration.

Post-hoc pairwise comparisons show several relevant patterns (Table 3A,B). The first is that hue exhibits more significant comparisons than the other color components. However, these significant differences involve only two of the subspecies contrasted against all (five) others. Subspecies *fulva* exhibits lower hue than other subspecies, and subspecies *sialis* shows higher hue than most. So hue ultimately is a contrast between two extremes and does not differentiate the other five subspecies. Chroma, on the other hand, differentiates between four of the seven subspecies, with two subspecies exhibiting low chroma and two higher chroma (Table 3). Subspecies *bermudensis* and *fulva* compared to *sialis* accounted for both instances where brightness differed. However, brightness shows the least number of differences among subspecies. The color component with the greatest number of differences among different subspecies is percent UV. There are two subspecies that show significantly lower percent UV than all other subspecies, and two subspecies with higher percent UV relative to the others. In addition *bermudensis* and *fulva* show highly divergent values for this component (Figure 3A, B). In most cases female color components (Table 3B) show the same patterns as males except where they add several additional contrasts between subspecies, such as between *meridionalis* and *fulva*. In general, *bermudensis*, *episcopus*, and *fulva* appear to be most divergent in overall coloration from the remaining subspecies.

The DA clearly shows that color components do not contribute equally to differences in plumage coloration across subspecies. The extent that each helps to differentiate between groups is heavily skewed towards percent UV as the strongest differentiator followed by chroma and then hue (Table 4). Brightness is a weak differentiatior relative to the other components. Both sexes show the same pattern with the exception of brightness on the second discriminant function axis (Table 4). Brightness in females has less explanatory power that brightness in males. In general, our DA results show that those color components that involve more subspecies in statistically significant comparisons (Table 3A,B) also discriminate the most between subspecies. In addition, the higher discriminatory power of the first axis is apparent in that more subspecies are resolved on this axis relative to the second discriminant axis (Figure 4A-D). Overall, the island population and the Mexican population that includes Arizona were most divergent in coloration from all other subspecies. The two southernmost Central American subspecies and the widespread North American subspecies were similar in coloration, countering our expectations for divergence across large geographic regions.

Discussion

By addressing variation in a color signal across multiple subspecies, we chose to confront a significant void in the literature [9]. Despite the numerous papers detailing individual variation in color components, none have specifically addressed how these same components vary across populations and subspecies [12,35-37]. The literature to date has been based on the way color components correlate with measures of fitness and breeding success [12,14]. We used an objective model of avian color perception to increase the

power of our inference by quantifying a color signal as it would be perceived by a bird. Our results provide a unique view of how plumage coloration varies across macro-scales, and allows us to derive more comprehensive expectations for how color may vary across multiple taxa. We show here that all components of blue structural coloration contribute to the variation in plumage across eastern bluebird subspecies. However, the majority of this variation occurs in color components that are condition dependent and thus prone to vary with ecological conditions. Furthermore, isolation by distance does not necessarily equate to greater differences in plumage color.

Is there a connection between patterns in color component variation at the individual-scale to that seen at the macro-scale? Out of the four components we measured in this study, percent UV differed most between bluebird subspecies. This color component is also known to show significant variation at the individual level [12,14]. Chroma is also known to exhibit high individual variability and this was the second best differentiator of subspecific groups in our analysis [26]. Therefore it may be that high component variability in individuals is a prerequisite for later differentiation across populations and subspecies.

In contrast, brightness had the smallest role in differentiating between subspecific coloration but is known to vary considerably with individual quality [12,34]. Why then does this component not contribute to differences between subspecies as do percent UV and chroma? Is it possible for variation in a color component to be restricted to the intraspecific level only? Brightness was not highly correlated with any one other color components and therefore it is unlikely to contribute any additive effects to variation in color. The brightness component of plumage is also processed independently from color

[15] and therefore may experience different selection pressures as an artifact of this difference in signal channel. This result suggests that we cannot assume all individual-level variability in color components scales up neatly to explain differences between populations and subspecies.

Hue is correlated with percent UV but not to the same extent that chroma and percent UV are related to each other. As a result, this color component is also less likely to show correlated change in response to modifications of other color components. This result is congruent with past research showing a stronger genetic component to the regulation of hue [14]. That same genetic component, however, implies that hue is less likely to vary between individuals and thus contribute to future differences between groups. That hue was strongly divergent in two subspecies relative to the other five is thus surprising and suggests that, in at least some eastern bluebird subspecies, hue is under strong directional selection.

Our results show that the path from one color phenotype to another can take numerous mechanistic routes. The different combinations of color components that diverge across eastern bluebird subspecies exemplify this pattern. There may ultimately be one path that is optimal, as in divergence along lines of chroma and percent UV. Perhaps this path happens with greater ease and may represent a distinct stage in the evolutionary process. Without a phylogeny of eastern bluebirds, it is impossible to determine if changes in percent UV and chroma occurred early in the differentiation of eastern bluebird subspecies, or if such changes in plumage color are a more recent event.

We have departed from past studies on color patterning and applied the analysis of geographic color variation to a detailed set of variables frequently overlooked by other

researchers. Furthermore, we have improved upon the description of intraspecific variation in color by generating objective measures of color created through a model of avian color vision [8]. As a result, we have extended our ability to test hypotheses on signal evolution by finding patterns in variation that can be explored across other taxa and regions. For example, do taxa like the orioles (Icteridae) show more variation in percent UV and chroma than they do in hue and brightness and how does variation in each component explain the remarkable diversity of coloration across the group? Our work suggests that, in general, avian lineages are likely to show small but measurable differences in hue, but that chroma and percent UV will explain most variation across a phylogeny. Not only are these patterns informative to our understanding of how plumage color evolves, but they are key to our understanding of other processes such as the development of premating barriers to reproduction [5] and the genetic control and expression of color [38]. By combining a detailed knowledge of variation in the color signal with variation in genes that control the expression of color, we stand to learn much about the generation of diversity in animal coloration.

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Table 1. Pearson correlation matrix for color variables across all subspecies. All correlations are significant at $p < 0.002$.

	Hue	Chroma	Brightness
Chroma	0.339		
Brightness	-0.339	0.410	
percent UV	0.616	0.827	0.212

Table 2. The ANCOVA results for each color component in males and females are depicted here with year and month as covariates and subspecies as the independent variable.

Dependent Variables	Independent Variables	F	DF	Coefficient	P
Male					
Hue (R^2 = 28.98)	Year	4.38	1	0.000561	0.038
	Month	0.58	1	0.002381	0.447
	Subspecies	13.27	6		0.000
Chroma (R^2 = 18.57)	Year	1.96	1	0.000171	0.163
	Month	8.85	1	-0.004247	0.003
	Subspecies	5.32	6		0.000
Brightness (R^2 = 17.34)	Year	1.19	1	-0.000199	0.276
	Month	5.96	1	-0.005192	0.016
	Subspecies	5.35	6		0.000
percent UV (R^2 = 17.20)	Year	8.05	1	0.000311	0.005
	Month	4.58	1	-0.002735	0.034
	Subspecies	4.72	6		0.000
Female					
Hue (R^2 = 30.44)	Year	0.02	1	-0.000082	0.890
	Month	2.67	1	0.012011	0.105

	Subspecies	8.90	6		0.000
Chroma (R^2 = 26.52)	Year	0.29	1	0.000063	0.594
	Month	0.60	1	-0.001120	0.441
	Subspecies	7.06	6		0.000
Brightness (R^2 = 9.64)	Year	1.09	1	-0.000230	0.299
	Month	0.01	1	-0.000213	0.938
	Subspecies	2.17	6		0.050
percent UV (R^2 = 17.20)	Year	8.05	1	0.000105	0.375
	Month	4.58	1	-0.000897	0.538
	Subspecies	4.72	6		0.002

Table 3A - D. Pairwise comparisons are shown between each subspecies for each color component (P = 0.05). In each panel, males are above the diagonal and females are below the diagonal. See Figure 2 for magnitude and direction of significant differences.

a) Hue

	BM	**EP**	**FU**	**GR**	**GU**	**ME**	**SI**
BM							M
EP							M
FU	F			M	M		M
GR			F				
GU							
ME			F				M
SI			F				

b) Chroma

	BM	**EP**	**FU**	**GR**	**GU**	**ME**	**SI**
BM							
EP					M		M
FU					M		M
GR							
GU			F				
ME			F				
SI			F				

c) Brightness

	BM	**EP**	**FU**	**GR**	**GU**	**ME**	**SI**
BM							M
EP							
FU							M
GR							
GU							
ME							
SI							

d) Percent UV

	BM	**EP**	**FU**	**GR**	**GU**	**ME**	**SI**
BM			M				
EP					M		
FU	F				M		M
GR							
GU			F				
ME			F				
SI			F				

Table 4. Depicted below are the coefficients of linear discriminants from a discriminant analysis on the differentiation of subspecies. In this analysis, we calculated the contribution of each color component to subspecies differentiation.

	Male		Female	
	LD1	LD2	LD1	LD2
Hue	-23.1	-18.78	-12.4	-8.3
Chroma	-58.5	13.8	-59.5	31.0
Brightness	12.0	-22.5	2.0	-18.0
percent UV	73.2	59.2	72.2	49.0
% Variance	75.4	15.7	68.1	24.6

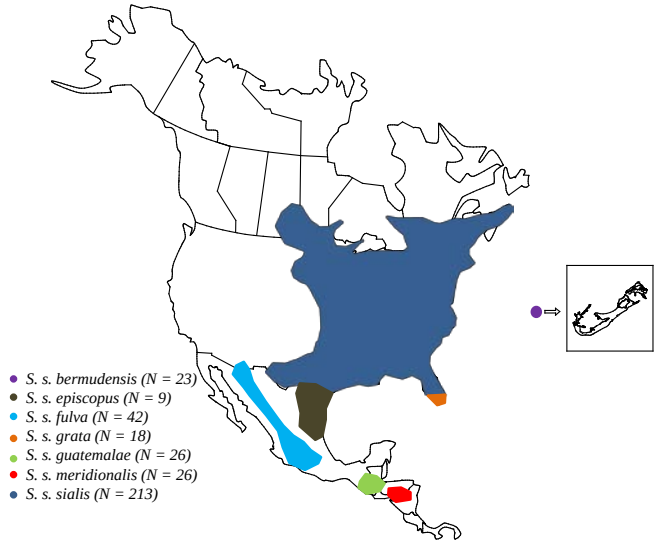


Figure 1. Map of eastern bluebird (*Sialia sialis*) distribution including subspecies and sample sizes.

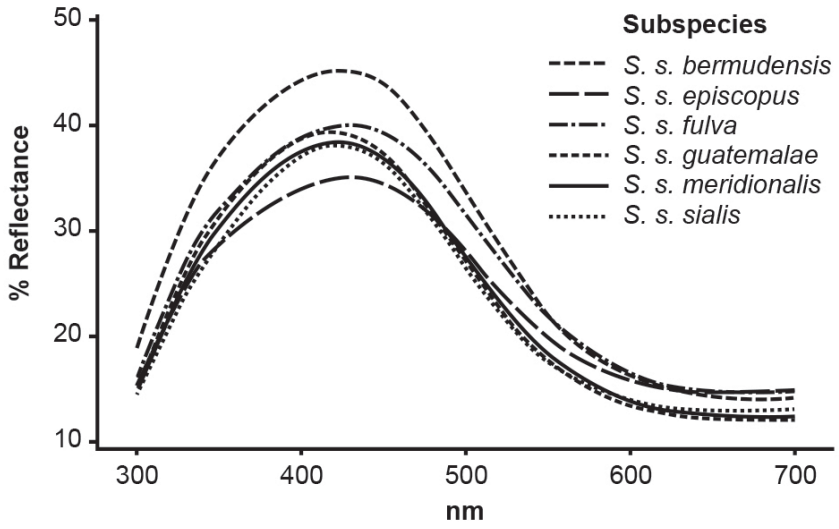
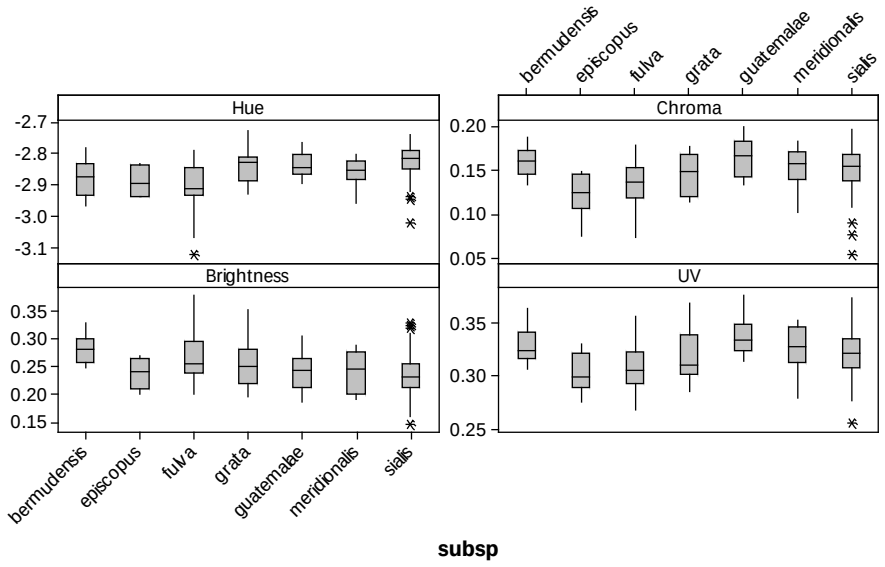


Figure 2. Average reflectance spectra for all subspecies with the exception of *S. s. grata* which was indistinguishable from *S. s. sialis*. Note the greater overall reflectance (brightness) in *S. s. bermudensis* and shift in wavelength of peak reflectance (hue) for *S. s. episcopus*.

A



B

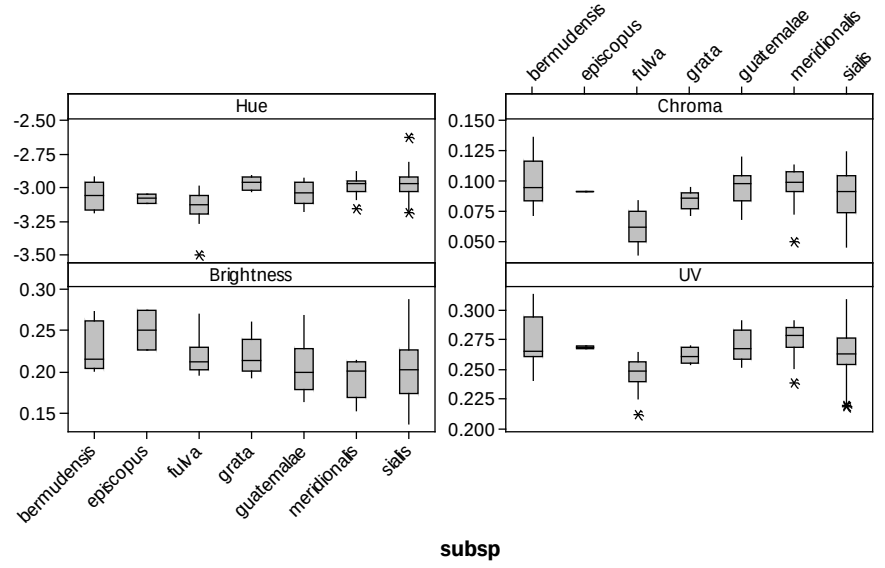
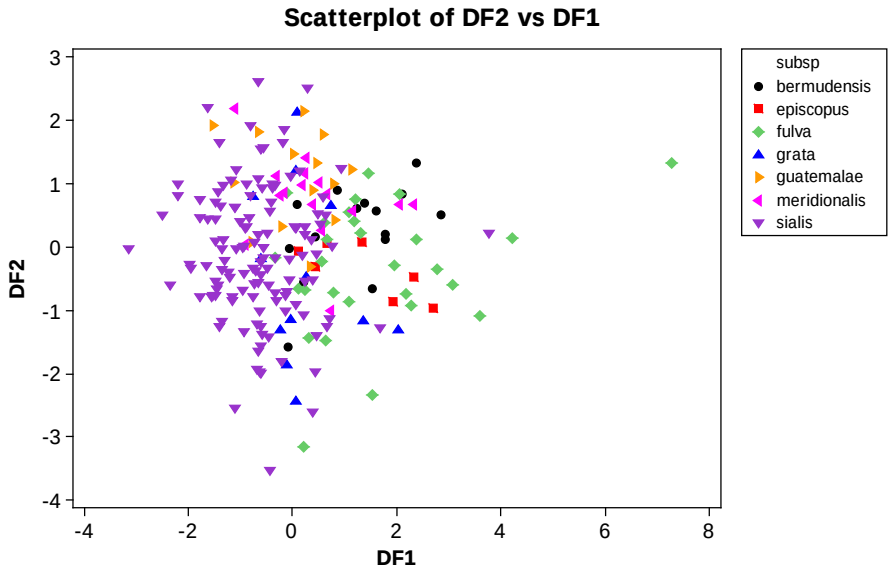
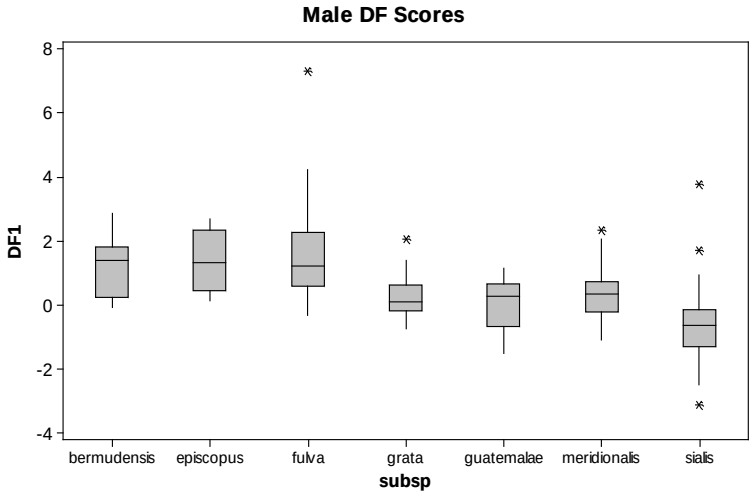


Figure 3A,B. The distribution of individual color components is shown for each subspecies in the figures above. Panel A depicts males and panel B depicts females.

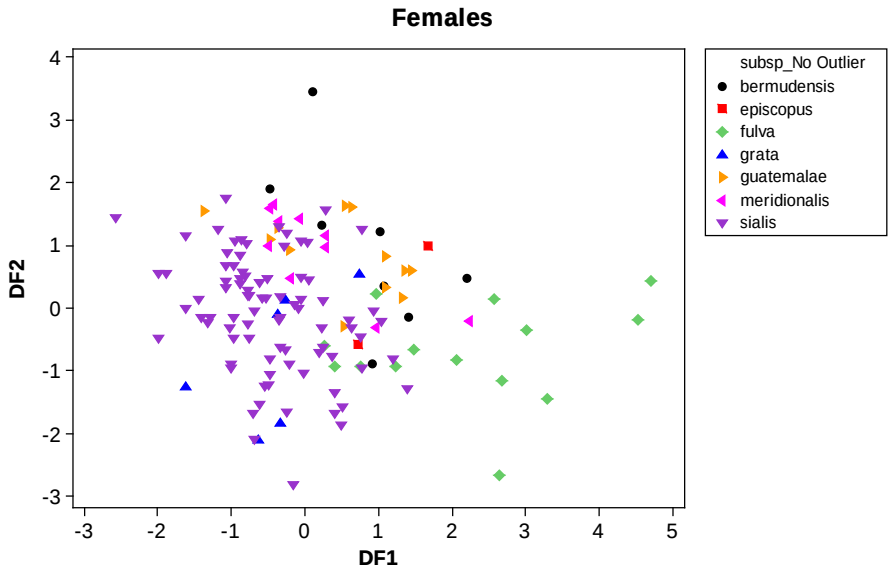
A


Scatterplot of DF2 vs DF1
DF2
DF1
subsp
bermudensis
episcopus
fulva
grata
guatemalae
meridionalis
sialis

B


Male DF Scores
DF1
bermudensis
episcopus
fulva
grata
guatemalae
meridionalis
sialis
subsp

C



D

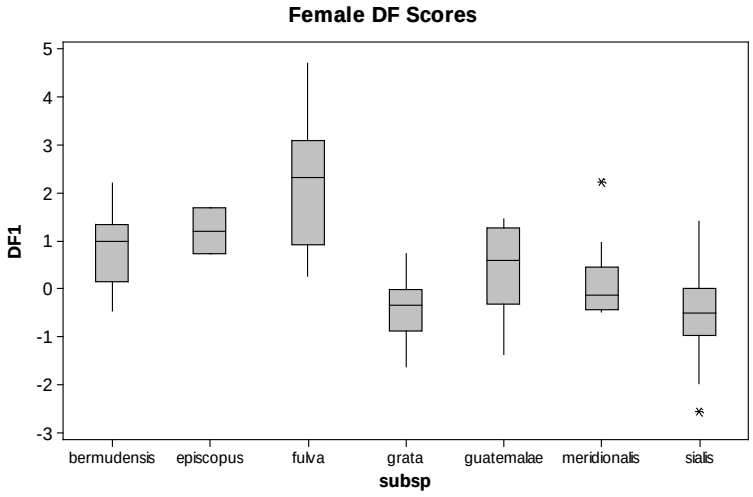


Figure 4A-D. Scatterplots showing discriminant function scores DF1 versus DF2 for both males and females along with boxplots showing the distribution of DF1 scores for each sex.

Concluding Remarks

In my research, I have explored a little understood island population of the eastern bluebird in order to progress our understanding of cryptic diversity and the evolution of animal coloration. I have found that humans potentially play a significant role in the undocumented movement of organisms. I have also found that plumage evolution on islands can take unexpected turns, differing with regard to several general expectations for color on islands. These general patterns in plumage coloration on islands can then be used to inform the study of broad-scale variation in color. At broad scales I found a disproportionate effect of several color components on differences in plumage color between subspecies. This pattern was also not always in the expected direction.

My finding that the eastern bluebird was likely introduced to Bermuda by humans exposes a problem that has not been fully acknowledged in the literature. The distribution of species on islands has been used to inform many biogeographical processes, and these distributions are assumed to be natural. At most we need to interpret contemporary animal and plant distributions with care, understanding that current patterns could have been influenced directly or indirectly by human activity. Furthermore, multiple lines of evidence should be used to interpret species distributions, as illustrated by the subspecific status of bluebirds on Bermuda. It is clearly possible for relatively young species to evolve significant differences in phenotype, and these trait divergence alone should not be used to label something as endemic.

My investigation of island bird plumage illustrates several intriguing findings. It is possible for newly isolated bird populations to exhibit strongly divergent coloration when compared to their mainland counterparts. Furthermore, I quantify for the first time

an exception to the rule with increased ornamentation and sexual dichromatism on an island. This change in coloration took place in two components of the color signal, hue and brightness. This is surprising because hue is thought to be relatively invariant and to signify an individual's genetic fitness, whereas brightness is highly variable at the intraspecific level and is known to be condition dependent. Variation in these two traits seems to be at odds with one another, particularly given my findings from the final chapter.

In the final section I show how color signal components do not contribute equally to differences between subspecies. In fact, there was a strong tendency for percent UV and chroma to contribute the most to differences between subspecies. The fact that brightness contributed very little to the differentiation of populations is surprising, given that it is highly variable across individuals. However, it may be that high individual variability in some traits is a prerequisite for future changes between populations and species. This supposition is supported by my observation that hue, a color component with a strong genetic predisposition, did not contribute as much to differences between subspecies as percent UV and chroma.

These results highlight the value of island species to the study of evolutionary and ecological processes. I show unequivocally that humans can shape communities in unexpected ways. In addition, I believe we now have some reasonable expectations for the manner in which avian species progress from one color plumage to another. I show a greater role for subspecies differentiation in the color components percent UV and chroma. It remains to be seen if this pattern holds for other taxa and other color producing mechanisms.

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2006	Curatorial Assistant, American Museum of Natural History, NY, Herpetology
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