## DISSERTATION

# MICROGEOGRAPHIC DIVERGENCE IN A SINGLE-ISLAND ENDEMIC: EVOLUTIONARY PATTERNS AND CONSERVATION IMPLICATIONS

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2014

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## ABSTRACT

## MICROGEOGRAPHIC DIVERGENCE IN A SINGLE-ISLAND ENDEMIC: EVOLUTIONARY PATTERNS AND CONSERVATION IMPLICATIONS

Understanding the processes that generate biological diversity is the central goal of evolutionary biology. Geographic isolation has traditionally been viewed as the primary scenario favoring evolutionary divergence. However, there is growing appreciation for the role of ecological variation and natural selection in driving adaptive differentiation, even in the absence of geographic barriers to gene flow. My dissertation tests for microgeographic patterns of local adaptation within one of the most range-restricted bird species in North America, the Island Scrub-Jay (Aphelocoma insularis). The species is restricted to Santa Cruz Island in southern California, USA, where it occurs in a diversity of habitat types within its narrow (250  $\text{km}^2$ ) geographic range. Remarkably, I found that Island Scrub-Jays in three separate stands of pine habitat had longer, narrower bills than those in oak habitat, a pattern that mirrors adaptive differences between allopatric populations of the species' mainland congener (A. californica). Adaptive divergence was not constrained by genetic diversity, even though Island Scrub-Jays had much lower levels of neutral genetic diversity than A. californica. Genetic data indicate that Island Scrub-Jays in pine habitat were more closely related to individuals in adjacent oak habitat than to individuals in other pine stands, indicating that each pine stand can be considered an independent case where adaptive divergence has been maintained in the face of some gene flow with adjacent oak birds. Morphological differences were not abrupt across the pine-oak boundary, as bill length declined gradually with distance from pine habitat, a clinal pattern that is also consistent with a scenario of divergence-with-gene flow. Individuals mated non-randomly with respect to bill length within the population, which may be due to a combination of (1) isolation-by-distance (localized dispersal) across the landscape and spatial autocorrelation in bill morphology, and (2) assortative mating at a more local scale based on bill morphology or correlated acoustic signals. These findings provide strong support for the notion that microgeographic patterns of local adaptation may be more common than is currently appreciated, even in mobile taxonomic groups like birds. They also underscore the importance of conserving Island Scrub-Jays across the entire island in order to preserve the species' full range of biological diversity and to facilitate adaptive responses to future environmental changes.

#### ACKNOWLEDGEMENTS

I am grateful to my advisors – Cameron Ghalambor and Scott Sillett – for their support and guidance on every aspect of this research project. Together they nurtured my dual interest in evolutionary biology and population ecology, and this dissertation certainly would not have been possible without their help. I am also thankful for the advice I received from my other committee members: Chris Funk, who provided expertise in evolutionary biology and genetics; Kevin Crooks, who provided expertise in ecology and conservation biology; and Scott Morrison, who helped to keep me grounded in the applied aspects of my research. This dissertation benefitted greatly from daily interactions with many lab-mates over the years: Michelle Desrosiers, Julián Torres Dowdall, Maybellene Gamboa, Corey Handelsman, Meribeth Huizinga, Emily Ruell, Alisha Shah, Helen Sofaer, and Jongmin Yoon. I am also extremely grateful for the feedback and advice I received from Lisa Angeloni, Vickie Bakker, Craig Benkman, Reed Bowman, John Fitzpatrick, Peter Grant, Rosemary Grant, Russ Greenberg, John McCormack, and Mike Webster.

My fieldwork would not have been possible without the support of many organizations on Santa Cruz Island and in Ventura, California. The Nature Conservancy and Channel Islands National Park permitted access to the entire island and provided logistical support and housing. The University of California Natural Reserve System's Santa Cruz Island Reserve provided office space, access to 4x4 vehicles (notably the trusty "Aphelocomobile"), and an opportunity to interact with many interesting visitors to the island. I would have been unable to obtain samples from the remote northern part of the island without helicopter transportation provided by Native Range. The whale-watching company Island Packers provided transportation to and from the island, and the Bella Maggiore Inn in Ventura provided a welcoming and comfortable place to

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stay during trips to the mainland. Many individuals at those organizations helped me along my path towards writing this dissertation, notably Jennifer Baker, Christie Boser, David Dewey, Kate Faulkner, Brian Guerrero, John Knapp, Lyndal Laughrin, Scott Morrison, Monica Vega, and Lotus Vermeer.

I am grateful to many funding sources for facilitating this work. My research was funded by a Student Research Award from the American Ornithologists' Union, a Chapman Research Award from the American Museum of Natural History, a Doctoral Dissertation Improvement Grant from the National Science Foundation, the Smithsonian Institution (Ornithology Student Research Award and other sources), The Nature Conservancy, Channel Islands National Park, and Colorado State University. I was also supported by a Graduate Research Fellowship from the National Science Foundation, a Jean Royce Alumni Fellowship from Queen's University, a Predoctoral Fellowship from the Smithsonian Institution, teaching assistantships from Colorado State University, and research assistantships from the Smithsonian. Finally, I would like to thank the Biology Department, the Graduate Degree Program in Ecology, the Ornithological Societies of North America, and the Canadian Society for Ecology and Evolution for providing travel grants that allowed me to present my dissertation research at scientific meetings.

Many people provided training and helped to collect data and samples. I am particularly grateful to the other graduate students on the Island Scrub-Jay project – Luke Caldwell, Michelle Desrosiers, and Mario Pesendorfer – for our collaborative efforts organizing field seasons, collecting data, and asking questions about our favorite study species. Fieldwork during the fall, when most of the data presented here were collected, was assisted by Sinéad Borchert, Elizabeth Donadio, Cassidy Grattan, Claire Guiliano, Justin Houck, Juan Klavins, Jessica Piispanen, Carolyn Sedgwick, and Colin Wooley. I would also like to thank Adam Dillon and his assistants

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for keeping an eye out for Island Scrub-Jay feathers while hiking between fox traps. At Colorado State University, several undergraduate students provided assistance with computer and laboratory work: Jessica Bender, Summer Grimes, Nevin Klein, Stacia Near, and Marina Rodriguez. This work was also made possible by molecular training from Melanie Murphy and recording-technique advice from Jenélle Dowling and Matthew Medler.

On a more personal note, I could not have completed this dissertation without the support of my family and friends. I owe my mother a particular debt of gratitude for nurturing my writing at a young age; she edited countless essays during my formative years, and any writing ability that I possess is in large measure due to her genetic contribution and tutelage. Finally, I would like to thank Dirk Hobman for keeping my spirits up while writing this dissertation; his smiling face was always a welcome relief during stressful times.

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## CHAPTER 1. General introduction

## Background

Population comparisons across environmental gradients have served a foundational role in our understanding of evolutionary processes. Nearly all species are exposed to heterogeneous ecological conditions and divergent selective pressures across their geographic range, leading to the prediction that populations may evolve traits that are best suited to the local environment (Kawecki & Ebert 2004). Examples of local adaptation abound, from the evolution of heavy metal tolerance in plants exposed to mine tailings (Macnair 1987), to the evolution of life-history strategies in guppies subject to varying levels of predation risk (Reznick et al. 1990; Torres Dowdall et al. 2012), to the evolution of coloration patterns in moths exposed to different soot concentrations in the environment (Kettlewell 1955). However, there are also examples where natural selection has failed to generate adaptive differences (Riechert 1993; Raymond & Marquine 1994; Postma & van Noordwijk 2005). Many factors can constrain the evolution of local adaptation (e.g., lack of heritability), but gene flow stands out as the primary microevolutionary force that acts in opposition to natural selection (Slatkin 1987; Lenormand 2002). Its role is not straightforward, however, and the complex interplay between gene flow and natural selection has generated fierce debate over the ecological and geographic conditions that are necessary for adaptive divergence to occur (Mayr 1947; Maynard Smith 1966; Schluter 2001; Gavrilets 2004; Räsänen & Hendry 2008). Debate continues to the present (Bolnick & Fitzpatrick 2007; Richardson et al. 2014), underscoring the need for more empirical work to understand the conditions that lead to evolutionary divergence when gene flow and divergent selection are acting simultaneously.

The necessity of geographic isolation has been central to debates on the mechanistic basis of local adaptation and speciation. Charles Darwin ascribed an important role for geographic isolation, but he also believed that natural selection could be powerful enough to generate adaptive differences within a single population (Darwin 1872, p. 82; Darwin 1876). After The Origin was first published in 1859 he corresponded with Moritz Wagner, who argued that geographic isolation was an essential component of the speciation process because adaptive differences could not be maintained in the face of continued migration (Wagner 1868). The scientific community largely sided with Darwin – viewing natural selection as the primary driving force behind evolutionary diversification – and debate on the topic subsided until the mid 20<sup>th</sup> Century, at which point Ernst Mayr resurrected many of the arguments put forward by Wagner. For Mayr, the crux of the problem boiled down to determining what mechanism was responsible for reducing gene flow between groups during the initial stages of divergence (Mayr 1947, 1963). He believed that geographic isolation (i.e., allopatric divergence) was the only plausible mechanism, at least for sexually-reproducing taxa, and he argued strongly against ecological scenarios that were hypothesized to generate patterns of non-random mating within populations exposed to disruptive selection pressures (e.g., assortative mating, imprinting on natal habitat; Mayr 1947). In the 1960s, a handful of papers were published questioning Mayr's view, based on theoretical models of disruptive selection (Maynard Smith 1966), empirical data on *Rhagoletis* flies (Bush 1969), and a laboratory experiment on *Drosophila* flies (Thoday & Gibson 1962). These studies were viewed with skepticism, however, and the prevailing view for several decades was that divergence in the absence of geographic barriers (i.e., sympatric divergence) was exceedingly rare in animal taxa, especially in the context of speciation

(Futuyma & Mayer 1980; Felsenstein 1981). It was not until the late 20<sup>st</sup> Century that enough evidence accumulated to overturn some of Mayr's arguments (Bush 1994; Via 2001).

One of the major lines of evidence against Mayr's position involved theoretical models that demonstrated the plausibility of divergence within a single population, most of which focused on the issue of recombination (Bolnick & Fitzpatrick 2007). Mayr (1963) argued that recombination in a randomly-mating population would prevent linkage disequilibrium from developing between traits under disruptive natural selection and traits related to mate choice, thus minimizing the potential for the evolution of non-random mating and adaptive divergence. However, an increasing number of studies have outlined ecological and genetic mechanisms for overcoming the issue of recombination in populations that lack physical barriers to gene flow. Most models start with the assumption that disruptive natural selection occurs within a single population because there are distinct habitat types (e.g., host plants in the case of phytophagus insects) or because there is selection for individual specialization along a continuous ecological gradient (e.g., prey size in the case of fish; Bolnick & Fitzpatrick 2007). From there they diverge in how they model mate choice, forming three broad groups:

 "Magic" trait models (Gavrilets 2004) assume that the trait under natural selection is directly related to mate choice, a scenario that invokes pleiotropy and thus bypasses the problem of recombination entirely. A number of traits have been suggested to act as "magic" traits (Servedio *et al.* 2011), including bill morphology in birds (Podos 2001; Derryberry *et al.* 2012), wing coloration in Lepidoptera (Jiggins *et al.* 2001; Fordyce *et al.* 2002), and body size and shape in fish (Nagel & Schluter 1998; Langerhans *et al.* 2007).

- One-allele marker trait models (Felsenstein 1981) assume that an allele arises at a separate, unlinked locus that leads to non-random mating within the population for the trait under disruptive natural selection. This could occur through the evolution of habitat selection based on an individual's natal origin or phenotype (Garant *et al.* 2005; Edelaar *et al.* 2008), the evolution of reduced dispersal on a heterogeneous landscape (Mallet *et al.* 2009), or the evolution of stronger assortative mating based on the ecological trait (Dieckmann & Doebeli 1999).
- 3. Two-allele marker trait models (Felsenstein 1981) assume that two ecologicallydivergent groups become fixed for different alleles at a separate, unlinked locus that determines mate choice. This mode of sympatric divergence is considered to be less likely than the others (Bolnick & Fitzpatrick 2007), but could be plausible if genetic drift causes the build-up of linkage disequilibrium between a locus under disruptive selection and a locus related to mate choice (Dieckmann & Doebeli 1999) or if there is multifarious (i.e., parallel natural and sexual) selection (Rice & Hostert 1993).

Most models have focused on the evolution of complete reproductive isolation (i.e., speciation), assuming a simple genetic architecture. However, other models have considered more complicated scenarios. For instance, Kondrashov & Kondrashov (1999) examined the evolution of quantitative traits and demonstrated that covariance between an ecological trait and a mating trait could evolve within a single population, even for quantitative traits that differ in their underlying genetic architecture. In another study, Bolnick (2006) included consideration of divergence at the intra-specific level and found that stable polymorphisms and partial reproductive isolation could result from a "magic" trait model if non-random mating was insufficiently strong.

In concert with the development of theoretical models, a growing number of empirical studies have found support for the plausibility of sympatric divergence. In a review of laboratory experiments, Rice & Hostert (1993) concluded that there was strong evidence that reproductive isolation could evolve within a single population subject to disruptive natural selection, especially when selection was multifarious (e.g., parallel selection on morphology and phenology). There are also many empirical examples where the process is thought to have occurred in nature. Some of the more notable examples of sympatric speciation involve host shifts in phytophagus insects (Wood & Keese 1990; Prowell et al. 2004) and brood-parasitic birds (Sorenson et al. 2003), as well as divergence within isolated environments on islands (Ryan et al. 2007; Friesen et al. 2007; Papadopulos et al. 2011) and in lakes (Schliewen et al. 1994; Rogers & Bernatchez 2007). At the intra-specific level, a number of studies have also documented the existence of sympatric ecotypes within species that are exposed to heterogeneous ecological conditions at fine spatial scales (e.g., Feder et al. 1988, Blondel 1999, Emelianov et al. 2004, Nosil et al. 2005, Huber et al. 2007). For many of these examples, the sympatric-origin claim has been questioned because of challenges associated with excluding the possibility of multiple colonization events and initial divergence in allopatry (Grant & Grant 2010). Nevertheless, the bulk of evidence has convinced most skeptics that sympatric divergence is at the very least a plausible evolutionary process (Coyne & Orr 2004).

The focus has now turned to understanding how frequently sympatric divergence occurs in nature and what mechanisms are involved (Bolnick & Fitzpatrick 2007; Richardson *et al.* 2014). The vast majority of empirical research on adaptive evolutionary divergence has been conducted at spatial scales that exceed the dispersal range of the focal species, in part because of historical arguments put forward by Mayr and others (Mayr 1963; Futuyma & Mayer 1980;

Felsenstein 1981) but also because a positive result (i.e., a finding adaptive divergence) is more likely at that spatial scale (Coyne & Orr 2004). A handful of empirical examples indicate that adaptive divergence at microgeographic scales (i.e., scales that correspond to the dispersal neighborhood of the focal species) may be more common than previously assumed, leading to a recent call for evolutionary biologists to reevaluate the spatial scale of adaptation (Richardson *et al.* 2014). Sympatric species pairs pose research challenges because of the difficultly associated with reconstructing the events that initially facilitated divergence; therefore, Bolnick & Fitzpatrick (2007) argued that more studies need to be designed to test for adaptive divergence within populations that are exposed to disruptive/divergent selection pressures. Theoretical work has marshaled many hypotheses to account for evolutionary diversification within a single population, and the current research priorities involve (1) testing the plausibility of the model assumptions, and (2) determining which mechanisms are most important for facilitating divergence in sympatry (Via 2001; Bolnick & Fitzpatrick 2007; Richardson *et al.* 2014).

My dissertation tests for adaptive evolutionary divergence within one of the most narrowly-distributed bird species in North America. Allopatry is widely considered to be the predominant mode of evolutionary diversification in birds (Mayr 1963; Coyne & Price 2000; Phillimore *et al.* 2008), in part because many species exhibit high rates of gene flow (Morjan & Rieseberg 2004). However, a growing number of studies on birds have found empirical support for divergence in sympatry (e.g., Blondel 1999, Sorenson *et al.* 2003, Ryan *et al.* 2007, Friesen *et al.* 2007, de León *et al.* 2010), raising the possibility that divergence-with-gene-flow may be more common in this highly-mobile group than is currently assumed. My focal species – the Island Scrub-Jay (*Aphelocoma insularis*) – is exposed to spatial heterogeneity in environmental conditions, but previous studies assumed that the species is phenotypically and genetically

homogeneous owing to its incredibly small (250 km<sup>2</sup>) geographic range (Pitelka 1951; Delaney & Wayne 2005). Here I document a surprising degree of spatial variability in phenotypic traits and neutral molecular markers within the Island Scrub-Jay, and evaluate conditions that may have facilitated microgeographic adaptation in this narrowly-distributed species.

### **Study system**

New World Jays in the genus Aphelocoma are a model system for the study of evolutionary diversification, as there are a wealth of examples documenting intra- and interspecific variation in ecological niche space (Rice et al. 2003; McCormack et al. 2010), social and breeding systems (Woolfenden & Fitzpatrick 1984; Berg et al. 2011), and morphological traits (Pitelka 1951; Peterson 1993; McCormack & Smith 2008). This clade of birds is relatively unusual in that there is no evidence for phylogenetic constraints on niche space, indicating an extreme degree of niche lability through evolutionary time (Rice et al. 2003). Aphelocoma populations occupy habitats that vary widely with regard to vegetation density (e.g., cloud forest vs. open scrub), the type of dominant vegetation (e.g., oak versus pine), and moisture levels (e.g., mangrove swamp vs. desert; Woolfenden & Fitzpatrick 1996; Curry & Delaney 2002; Curry et al. 2002; McCormack & Brown 2008). They also exhibit morphological adaptations that are associated with transitions between habitat types (Peterson 1993; Bardwell et al. 2001; McCormack & Smith 2008). For instance, populations of Western Scrub-Jay (A. californica) that occur in pine habitat across the interior of North America have longer, straighter bills than populations that occur in oak habitat along the coast (Peterson 1993). These morphological differences are adaptive because long, narrow bills are more efficient for feeding on pine cones and short, stout bills are more efficient for feeding on acorns (Bardwell et al. 2001).

The Island Scrub-Jay is the most narrowly-distributed species of *Aphelocoma* jay (Pitelka 1951). The entire global population is restricted to Santa Cruz Island, which is a small (250 km<sup>2</sup>) but ecologically-heterogeneous island that is located 30 km from the mainland of southern California (Curry & Delaney 2002; Schoenherr *et al.* 2003). The insular species is most closely related to Californian populations of the Western Scrub-Jay (*A. californica*; Delaney *et al.* 2008; McCormack *et al.* 2011). However, Island Scrub-Jays are much larger and brighter than their mainland relatives and molecular data indicate that they have been evolving in isolation for approximately one million years (McCormack *et al.* 2011), with no evidence of ongoing dispersal or gene flow with mainland populations (Delaney & Wayne 2005; Collins 2009).

Island Scrub-Jays occupy a variety of environmental conditions despite being confined to a single island. The two main vegetation communities used by the species are: (1) oak chaparral and woodland, which is dominated by the Island Scrub Oak (*Quercus pacifica*) but also includes other oak species (*Q. agrifolia*, *Q. tomentella*, *Q. parvula*, *Q. chrysolepis*, *Q. douglasii*, *Q. lobata*, and *Q. macdonaldii*), and (2) Bishop Pine (*Pinus muricata*), a closed-cone pine that is restricted to areas with significant inputs of marine fog (Junak 1995). Oak forms the primary matrix of jay habitat across the landscape (covers 43% of the island versus 8% pine), bisecting three pine stands in the western, central, and eastern portions of the island (see Fig. 1.1). However, during the cooler and wetter Pleistocene, coniferous forests predominated across the northern Channel Islands (Schoenherr *et al.* 2003; Anderson *et al.* 2009), so the current pine stands on Santa Cruz Island can be viewed as relicts of what was once a much more continuous distribution.

Island Scrub-Jays occur at much higher densities than Western Scrub-Jays in similar habitat on the mainland (Yeaton 1974). Both species form monogamous social pairs and are

highly territorial, defending multi-purpose territories throughout the year (Curry & Delaney 2002; Curry *et al.* 2002). However, Island Scrub-Jays have smaller territories (Carmen 2004; Caldwell *et al.* 2013) and there is less turnover because territorial individuals have higher rates of annual survival (Atwood *et al.* 1990). Much of the habitat available for Island Scrub-Jays on Santa Cruz Island appears to be saturated, a situation that forces many individuals to exist as non-breeding "floaters" for several years until they can acquire a breeding territory (Collins & Corey 1994). Few data are available on the pair-formation process in Island Scrub-Jays, so it is unclear whether individuals acquire territories through competitive interactions (e.g., male-male competition) or active mate selection (e.g., widowed bird selecting mate) when vacancies arise.

Island Scrub-Jays have been identified as a species of conservation concern (Morrison *et al.* 2011). Historical data indicate that Island Scrub-Jays were extirpated from a neighboring island (Santa Rosa) in the past 1000 years (Collins 2009), highlighting the vulnerability of the only extant population on Santa Cruz Island. The species has a census population size of fewer than 3000 individuals (Sillett *et al.* 2012), and it was recently uplisted to "vulnerable" on the IUCN Red List because of its narrow geographic range and concerns about the potential for short-term threats like fire and the arrival of West Nile Virus (IUCN 2012). Current ownership of Santa Cruz Island is in the hands of The Nature Conservancy and Channel Islands National Park, organizations that have taken a proactive approach to managing conservation threats faced by Island Scrub-Jays (Morrison *et al.* 2011). My dissertation was designed to dovetail with their conservation priorities, so the research presented here not only addresses broad questions in evolutionary biology, using the Island Scrub-Jay as a model system, but also has implications for the conservation and management of this rare island endemic.

### **Research overview**

Genetic variation is a prerequisite for adaptive evolutionary divergence (Allendorf *et al.* 2012). In this respect, populations that are endemic to islands are an evolutionary conundrum. Many have exceptionally low levels of genetic diversity owing to founder effects and chronic genetic drift (Frankham 2008). Yet, islands provide some of the most remarkable examples of adaptive evolutionary divergence (Schluter 2000), notably Darwin's finches on the Galapagos Islands (Grant & Grant 2011), *Anolis* lizards in the Greater Antilles (Losos 2009), and silverswords in Hawaii (Robichaux *et al.* 1990).

Chapter 2 aims to provide a basic understanding of factors that have shaped levels of neutral genetic diversity within the Island Scrub-Jay. Low genetic diversity could constrain the species' adaptive potential, and it could also have more immediate ramifications for Island Scrub-Jay conservation if there are negative fitness consequences due to inbreeding depression. My co-authors and I found that neutral genetic diversity was lower in Island Scrub-Jays compared to Western Scrub-Jays on the mainland of California. There was no evidence that anthropogenic habitat loss due to ranching activities in the 19<sup>th</sup> and 20<sup>th</sup> Centuries reduced genetic diversity below historical levels. However, genetic diversity did vary spatially, with lower levels towards the periphery of the island, indicating that microevolutionary processes (gene flow, genetic drift) have acted within this population to reduce the heterozygosity of some individuals. We detected few fitness costs of low genetic diversity: individuals that were relatively more inbred had a lower probability of acquiring a breeding territory early in life, but there was no evidence that low genetic diversity had a negative impact on survival or fecundity.

conservation threat to the Island Scrub-Jay, but this does not negate the importance of guarding against future losses of genetic diversity to maintain the species' adaptive potential.

In Chapter 3, my co-authors and I quantified gene flow across the range of the Island Scrub-Jay and tested for microgeographic adaptation in bill morphology. Island Scrub-Jays occur in oak and pine habitats on Santa Cruz Island, the same habitat types that were previously found to exert divergent selective pressures on bill morphology in allopatric populations of Western Scrub-Jays (Peterson 1993) due to tradeoffs in feeding efficiency on acorns vs. pine cones (Bardwell *et al.* 2001). The Island Scrub-Jay presents an ideal opportunity to test for parallel patterns of adaptive divergence within a single population because there are repeated oak-pine transitions at small spatial scales on Santa Cruz Island (see Fig. 1.1) – with no physical barriers to gene flow – and the limited insular range precludes the effects of gene flow from elsewhere, an issue that can confound studies of adaptive divergence (Postma & van Noordwijk 2005).

We detected genetic structure and spatially-replicated patterns of adaptive divergence within the Island Scrub-Jay, contrary to expectations that the species would be genetically and phenotypically homogenous owing to its narrow geographic range. Island Scrub-Jays in three separate stands of pine habitat had longer, narrower bills than jays in oak habitat, a pattern that mirrors morphological adaptations for nut consumption in Western Scrub-Jays. There was not a sharp transition in bill morphology between habitat types; instead bill length declined gradually with distance from pine habitat, a clinal pattern that is consistent with a scenario of adaptive divergence in the face of gene flow (May *et al.* 1975; Moore & Hendry 2005). Dispersal and gene flow appears to be localized in Island Scrub-Jays based on (1) demographic data on natal dispersal, and (2) genetic data that revealed a continuous pattern of isolation-by-distance along the east-west axis of the island. There was also evidence for a reduction in gene flow across the

boundary of the westernmost and largest pine stand, which could be due to selection against individuals that disperse between habitats, habitat selection based on natal origin, or active mate selection.

Consistent with models of sympatric divergence, we found that Island Scrub-Jays mated non-randomly with respect to bill morphology: at the scale of the island, long-billed males were more likely to form breeding pairs with long-billed females. There are two possible explanations for this pattern: (1) Island Scrub-Jays pair with nearby individuals, which happen to be morphologically similar because of localized dispersal and spatial autocorrelation in bill morphology (consistent with one-allele marker trait models), (2) Island Scrub-Jays actively select mates based on a mating cue that is related to variation in bill morphology (consistent with "magic" trait models). The first explanation likely accounts for a portion of the non-random mating we documented in Island Scrub-Jays. However, it is also possible that bill morphology is related to a cue used in mate choice. Previous work suggests that bird bills can function as "magic" traits because – in addition to being under strong selection for foraging on particular food resources – changes in bill morphology can cause correlated changes in vocalizations used during mate choice (Podos 2001; Ballentine 2006; Badyaev *et al.* 2008; Derryberry 2009; Derryberry *et al.* 2012).

In Chapter 4 my co-authors and I tested for a relationship between bill morphology and vocal signal structure, with the aim of examining whether the bill may be acting as a "magic" trait in Island Scrub-Jays. The acoustic signal we chose to focus on was the female-specific rattle call, which is a rapid, broad-band vocalization that is important for male-female interactions in *Aphelocoma* and other corvids (Goodwin 1976; Woolfenden & Fitzpatrick 1996; Curry & Delaney 2002). We found that females with longer bills produced calls that were more rapid, and

those with deeper bills produced calls that were higher in frequency. There was no relationship between vocal signal structure and habitat characteristics, indicating that this pattern cannot be attributed to acoustic adaptations for optimizing sound transmission. Instead, our results suggest that bill morphology may be acting as a "magic" trait in the Island Scrub-Jay. Further research is needed to understand the relationship between bill morphology and vocal signal structure in Island Scrub-Jays, and to determine how this linkage contributes to non-random mating within the population. Nevertheless, these findings provide empirical support for mechanisms that have been proposed to facilitate adaptive evolutionary divergence within populations exposed to divergent selection pressures.

## Significance

Collectively, these results reveal striking patterns of phenotypic and genetic variation within one of the most narrowly-distributed bird species in North America. Adaptive divergence was not constrained by genetic diversity, even though Island Scrub-Jays had much lower levels of neutral genetic diversity than their sister species on the mainland of California. Remarkably, the difference in bill length between pine jays and jays in oak >4 km distant from pine habitat was of a similar magnitude as the difference documented for allopatric populations of Western Scrub-Jays occurring in pine and oak habitat (Peterson 1993). These data provide strong support for the notion that microgeographic patterns of local adaptation may be more common than is currently appreciated (Richardson *et al.* 2014), even in mobile taxonomic groups like birds.

They also shed light on the conditions that may facilitate adaptive evolutionary divergence in sympatry. Island Scrub-Jays are distributed across a landscape that consists of a mosaic of different habitat types. These habitats generate divergent selection pressures that are

requisite for adaptive evolutionary divergence, but in addition to that they may have a spatial arrangement that is especially conducive to facilitating divergence. Mallet *et al.* (2009) recently argued that sympatric divergence is much more likely to occur when divergent habitats occur as a mosaic across the landscape, as opposed to a situation of pure sympatry (see Fig. 1.2). This is particularly the case for populations that exhibit a genetic pattern of isolation-by-distance, as non-random mating can occur as a by-product of spatial autocorrelation in selection pressures and limited dispersal across the landscape (Mallet *et al.* 2009). This process likely explains a substantial portion of the non-random mating for bill morphology in Island Scrub-Jays at the scale of the island. On top of that, there may be assortative mating on a more local scale that is driven by corresponding divergence in acoustic signals. A number of other factors may have facilitated the evolution and maintenance of divergence in sympatry, including strong selection, source-sink dynamics (with pine as the source habitat), competition for limited breeding vacancies, selection for reduced dispersal because of outbreeding depression, habitat-biased dispersal caused by imprinting on natal habitat, and the occurrence of ecological opportunity within a depauperate insular community. Ultimately, I hope that the data presented here provide the foundation for many subsequent studies aimed at understanding the mechanisms and implications of adaptive evolutionary divergence in Island Scrub-Jays.

#### **Conservation implications**

These findings also have implications for the conservation of the only bird species endemic to the California Channel Islands. In the past few decades, conservation biologists have increasingly focused on the importance of preserving biological diversity at the intra-specific level in order to maintain the ecological resilience and adaptive potential of species (Crandall *et* 

*al.* 2000; Hughes *et al.* 2008; Hoffmann & Sgrò 2011). The Island Scrub-Jay has lower levels of neutral genetic diversity than its closest relative on the mainland, which could be cause for concern because low genetic diversity is implicated as a factor that may lead to an increased risk of extinction (Frankham 2008). However, there is no evidence for strong inbreeding depression within the population, and Island Scrub-Jays appear to have maintained historical levels of genetic diversity despite the recent impact of ranching activities in the 19<sup>th</sup> and 20<sup>th</sup> Centuries. This has positive implications for the likelihood of long-term population persistence because historical levels of genetic diversity were sufficient to enable the species to survive (1) drastic environmental changes that accompanied the transition from the Pleistocene to the Holocene, and (2) habitat alterations during the more recent ranching era.

To ensure the species' resilience is maintained in the future, conservation efforts should focus on guarding against any losses of genetic diversity. This will require conserving Island Scrub-Jays in different regions of the island – and hence thinking about *management* at a microgeographic scale – because the population does not exhibit spatial homogeneity in adaptive traits and neutral genetic diversity. For instance, if West Nile Virus were detected on Santa Cruz Island, managers should inoculate Island Scrub-Jays across the entire island and they may also want to consider establishing multiple, spatially-representative captive populations (rather establishing a single captive population). Heterozygosity is lower towards the periphery of the island, likely due to increased genetic drift and reduced gene flow, so another potential management option could involve relocating Island Scrub-Jays within Santa Cruz Island (e.g., from the isthmus to the eastern side of the island). Dispersal in males is incredibly limited (< 1 km), so one strategy would be to relocate males to areas along the periphery of the island where habitat is currently regenerating. This could reduce the potential for the loss of genetic diversity

through genetic drift, and it may also have positive benefits for the population growth rate in those regions of the island if it increases the likelihood that dispersing females are able to find mates.

The data presented here may also be useful for developing a future plan for reintroducing Island Scrub-Jays to neighboring Santa Rosa Island (Morrison *et al.* 2011). Island Scrub-Jays appear to be locally adapted to different habitat types, and this could be the case for other ecological traits as well (e.g., climatic tolerance). Outbreeding depression could potentially be an issue if the individuals that comprise the re-introduced population were to originate from different regions of Santa Cruz Island. Therefore, managers may want to select individuals from the western portion of the species' range, which is more similar climatically to Santa Rosa Island. The areas where jays would likely be released on Santa Rosa Island contain oak (primarily *Q. agrifolia* and *Q. pacifica*) and pine (*P. muricata* and *P. torreyana*) trees; therefore, managers may also want to reintroduce a mix of individuals that originate from pine habitat and oak habitat > 4 km from pine to maximize the adaptive potential of the new population.

## Figures



**Figure 1.1** Map of Santa Cruz Island, which constitutes the entire geographic range of the Island Scrub-Jay. Colors represent habitat type (orange = oak chaparral and woodlands, green = pine forests, yellow = vegetation community lacking oak or pine trees), shading depicts topography, and the inset shows the location of the island relative to the mainland of southern California. Vegetation data are from The Nature Conservancy (2007) and the other spatial layers are from the Environmental Systems Research Institute (Redlands, California, USA).



**Figure 1.2** Visual representation of scenarios representing divergence between two groups (e.g., morphotypes, subspecies, species; represented by the circles and triangles) in (a) allopatry, (b) parapatry, (c) pure sympatry, and (d) mosaic sympatry [adapted from Mallet *et al.* (2009)].

CHAPTER 2. An evaluation of the causes and consequences of reduced genetic diversity in a single-island endemic<sup>1</sup>

## Summary

Many species that are endemic to islands have persisted for thousands of years, despite small population sizes, pronounced geographic isolation, and relatively low levels of neutral genetic diversity. This observation has challenged the prevailing view that small populations, especially those on islands, are vulnerable to extinction because of the ecological and evolutionary consequences of low genetic diversity. Here we examine factors that have shaped the genetic diversity of a single-island endemic species, the Island Scrub-Jay (Aphelocoma insularis), and test for evidence of inbreeding depression. Not surprisingly, we found that neutral genetic diversity was lower than in the species' closest relative on the mainland. There was no evidence that anthropogenic habitat loss during the 19<sup>th</sup> and 20<sup>th</sup> Centuries reduced genetic diversity below historical levels. However, genetic diversity did vary spatially, with lower levels towards the periphery of the island, indicating that microevolutionary processes (gene flow, genetic drift) have acted within this population to reduce the heterozygosity of some individuals. We detected few fitness costs of low genetic diversity: individuals that were relatively more inbred had a lower probability of acquiring a breeding territory early in life, but there was no evidence that low genetic diversity had a negative impact on survival or fecundity. These results indicate that inbreeding depression does not represent an immediate and pressing conservation

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threat to the Island Scrub-Jay, but this does not negate the importance of guarding against future losses of genetic diversity in this rare and geographically-restricted species.

## Introduction

The preservation of genetic diversity is an important conservation and management objective because it can reduce inbreeding depression (Hedrick & Kalinowski 2000), maintain ecological resilience (Hughes *et al.* 2008), guard against disease outbreaks (Spielman *et al.* 2004), and maintain evolvability in the face of environmental change (Sgrò *et al.* 2011). Unfortunately, the erosion of genetic diversity is a common side effect of many threats facing natural populations. For example, reductions in population size due to habitat loss can increase the strength of genetic drift, and reductions in connectivity due to habitat fragmentation can decrease the frequency at which new alleles are introduced to populations via gene flow (Allendorf *et al.* 2012). There is increasing evidence that low levels of genetic diversity can increase a species' risk of extinction (Frankham 2005; O'Grady *et al.* 2006); however, debate continues over whether this is the case for all taxa (Jamieson 2007).

A primary focus of this debate has involved taxa that are endemic to islands. Compared to continents, the extinction rate on islands is disproportionately high (Whittaker & Fernandez-Palacios 1999; Ricketts *et al.* 2005; Johnson & Stattersfield 2008) and many endemics that are currently threatened persist as small, isolated populations that have extremely low genetic diversity (e.g., Eldridge *et al.* 1999, Aguilar *et al.* 2004). Furthermore, the level of inbreeding necessary to elevate extinction risk (based on work with inbred lines of domestic and laboratory species; e.g., Eisen & Hanrahan 1974, Rumball *et al.* 1994) is on par with the level of inbreeding documented in many island populations, sparking concern that genetic factors have contributed

to the imperilment of species on islands (Frankham 2008). The other side of the argument hinges on the observation that many insular populations were founded by a small number of individuals and have persisted for thousands of years, despite small population sizes, pronounced geographic isolation, and low genetic diversity (Craig 1991, 1994). In fact, these factors may have made them more resistant to the negative effects of inbreeding: deleterious recessive alleles are more likely to be expressed in small, inbred populations, so selection may have already eliminated them through genetic purging (Wright 1977). One limitation of this argument is that the genetic basis of inbreeding depression is environmentally-dependent, so alleles that were previously neutral may be expressed as deleterious if the environment changes (Bijlsma *et al.* 1999). This may at least partially account for the fact that inbreeding depression has been observed in species that are endemic to islands (Eldridge *et al.* 1999; Keller *et al.* 2002; Jamieson *et al.* 2006), many of which have undergone environmental alteration from anthropogenic activity.

Here we ask whether genetic factors are a pressing conservation concern for a rare island endemic, the Island Scrub-Jay (*Aphelocoma insularis*), which has been isolated on the Channel Islands of southern California, USA, for approximately one million years (McCormack *et al.* 2011). The contemporary population is entirely restricted to Santa Cruz Island (hereafter Santa Cruz) and has an estimated census population size of fewer than 3000 individuals (Sillett *et al.* 2012), but there is evidence to suggest that the species was present on nearby Santa Rosa Island (hereafter Santa Rosa) at least 1000 years ago, and perhaps into the 19<sup>th</sup> Century (Collins 2009). Both islands were extensively de-vegetated by non-native ungulates [pigs (*Sus scrofa*) and cattle (*Bos taurus*) on both islands; sheep (*Ovis aries*) on Santa Cruz; black-tailed deer (*Odocoileus hemionus*) and elk (*Cervis elaphus*) on Santa Rosa] in the 19<sup>th</sup> and 20<sup>th</sup> Centuries (Schoenherr *et al.* 2003). Those animals have since been removed, but decades of overgrazing greatly reduced the amount of habitat available for Island Scrub-Jays (oak chaparral and pine woodland; Curry & Delaney 2002; Sillett *et al.* 2012) and enabled the expansion of grassland (Vuren & Coblentz 1987; Schoenherr *et al.* 2003). Island Scrub-Jays likely survived the ranching era on Santa Cruz because the island's rugged terrain allowed for the persistence of larger and more frequent patches of woody vegetation than on Santa Rosa (Collins 2009). Still, it has been hypothesized that the Island Scrub-Jay population on Santa Cruz declined during the ranching era (Vuren & Coblentz 1987; Morrison *et al.* 2011; Sillett *et al.* 2012).

The first objective of our study was to confirm that genetic diversity is lower in Island Scrub-Jays than in the species' mainland congener, the Western Scrub-Jay (*A. californica*). A previous comparison reported that Island Scrub-Jays had significantly lower levels of neutral genetic diversity (Delaney & Wayne 2005), but it was based on a small (n = 25), geographicallyrestricted sample of island birds. That sampling scheme is potentially problematic because recent work detected spatial genetic structure across the range of the Island Scrub-Jay, a pattern that is primarily driven by isolation-by-distance due to localized dispersal (Chapter 3). Therefore, we genotyped 563 Island Scrub-Jays that were sampled across Santa Cruz Island to generate a better estimate of relative levels of genetic diversity in Island Scrub-Jays.

The other objectives of our study involved testing hypothesized causes and consequences of reduced genetic diversity in Island Scrub-Jays. We hypothesized that a population decline during the ranching era reduced genetic diversity below historical levels, so we tested for the genetic artifacts of a recent population bottleneck. Given the lack of panmixia within the contemporary population, we also hypothesized that microevolutionary processes (genetic drift, gene flow) that influence genetic diversity vary spatially across the island, so we tested for spatial autocorrelation in the heterozygosity of individuals. Finally, we hypothesized that low

levels of genetic diversity would have negative fitness consequences for Island Scrub-Jays, especially if the population suffered a loss of genetic diversity during a recent bottleneck. Therefore, we tested for evidence of inbreeding depression using demographic data on territory acquisition, survival, and reproduction.

#### Methods

## Sample collection

We collected genetic material from ~25% of the contemporary population of Island Scrub-Jays (n = 563), a sample that includes individuals that were distributed across available habitat on Santa Cruz (Fig. 2.1). Genetic material was obtained by collecting blood from captured individuals (n = 544) and by collecting feathers that were found on the ground (n = 19). Most individuals were captured with baited drop-traps or mist-nets; they were then marked with a numbered aluminum leg band and a unique combination of plastic colored leg bands, aged using plumage characters (Pyle 1997), and sampled for blood (20-60 µL). Blood was stored in lysis buffer (0.1M Tris HCl, 0.1M EDTA, 0.01M NaCl, 0.5% SDS) until DNA extraction, and feather samples were stored in envelopes. These protocols were conducted in accordance with IACUC permits issued by Colorado State University (CSU) and the Smithsonian Institution (SI), a U.S. federal banding permit issued to the SI, and California scientific collection permits issued to CSU and the SI.

## Microsatellite genotyping

DNA was extracted from blood samples and pulp cells within the rachis of feather samples using a DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA), following

manufacturer protocols. All samples were then genotyped at 12 polymorphic microsatellite loci (15 alleles per locus, on average; see Table 2.1), which were originally developed for the following corvids: A. insularis [AIAAGG13 (Delaney & Wayne 2005)], A. coerulescens [ApCo2, ApCo30, ApCo37 (Stenzler & Fitzpatrick 2002)], A. ultramarina [MJG6 (Li et al. 1997)], Gymnorhinus cyanocephalus [PJGATA2, PJGATA3, PJAAAG9 (Busch et al. 2008)], and Psilorhinus morio [CmAAAG6, CmAAAG11, CmAAAG25, CmAAAG30 (Williams et al. 2004)]. Some of the loci (AIAAGG13, ApCo2, ApCo30, ApCo37, MJG6) were genotyped by Delaney & Wayne (2005); the others were selected by (1) screening loci that had been developed for other corvids and (2) identifying the ones that were the most polymorphic in Island Scrub-Jays (data were not collected for loci that were monomorphic or weakly polymorphic in our initial screening). Microsatellites were amplified in 10 µL multiplex reactions (with 2-5 loci per reaction; see Table 2.S1) using QIAGEN's type-it microsatellite PCR kit and a Vapo-Protect Mastercycler 6321 (Eppendorf, Hauppange, NY, USA). Loci in the same reaction were distinguished from one another by unique fluorescent labels or by pre-determined allele-size differences. PCR products were run on an Applied BioSystems 3730xl DNA Analyzer, and the resulting electropherograms were scored automatically and checked manually using GeneMarker (SoftGenetics, State College, PA, USA). Negative and positive controls were run with each batch of reactions to check for contamination and repeatability, respectively. The entire process from amplification to allele scoring was also repeated for 8% of the samples (n = 46) at all 12 loci, and we detected no discrepancies. The genotypes of feather samples were compared to the genotypes of blood samples collected in the vicinity to prevent duplicate sampling of the same individual.

## Demographic data

We tested for inbreeding depression using data that were collected on three demography plots (~500 ha total) in oak chaparral habitat from 2008 to 2013. The plots were located within 6 km of the center of the island [see map in (Caldwell *et al.* 2013)], where Island Scrub-Jay population densities are highest (Fig. 2.S1; Sillett *et al.* 2012). Individual jays were identified by unique leg band combinations and were intensively surveyed each spring and fall to collect data on: (1) territory status, by documenting all of the individuals that held breeding territories, (2) survival, by conducting surveys to resight marked birds within our study-plot boundaries (each plot was usually surveyed every 3 days), and (3) reproduction, by finding and monitoring nests during the breeding season. The sex of all individuals was determined through behavioral observations and molecular sexing (detailed in Chapter 3).

### Analyses

*Basic genetic analyses.* We tested for the presence of null alleles and large-allele dropouts using MICRO-CHECKER software (Van Oosterhout *et al.* 2004). Data for two loci not mentioned above [ApCo29 (Stenzler & Fitzpatrick 2002), MJG4 (Li *et al.* 1997)] were excluded due to evidence of null alleles. GENEPOP software (Raymond & Rousset 1995) was used to test for departures from Hardy-Weinberg equilibrium and linkage disequilibrium (within each of the three panmictic subregions shown in Fig. 2.1; see below for rationale on the use of those regions) and to calculate summary statistics for each locus. Three out of 36 Hardy-Weinberg tests were significant, which is close to the number expected based on chance. We also found no evidence that loci were consistently associated with each other in our tests for linkage disequilibrium, so we proceeded with analyses assuming independence among loci.

*Island-mainland comparison.* Five of the loci (ApCo2, ApCo30, ApCo37, AIAAGG13, and MJG6) we used to genotype Island Scrub-Jays were used in a previous study to genotype Western Scrub-Jays that had been sampled across mainland California (Delaney & Wayne 2005). We used paired t-tests [implemented in R (R Core Team 2012)] to determine whether populations of the two species in California differed in two measures of population-level genetic diversity: number of alleles per locus and observed heterozygosity [metrics that were both reported in Delaney & Wayne (2005)].

Temporal and spatial variation in genetic diversity. We used the heterozygosity-excess method described in Cornuet & Luikart (1996) to test for the genetic signature of a recent population bottleneck. This method is based on the premise that the loss of rare alleles during bottleneck events results in a negligible decline in heterozygosity; as a result, heterozygosity in a recently-bottlenecked population should be higher than expected based on mutation-drift equilibrium. Simulations have shown that this method has lower type I and II error rates compared to other single-sample bottleneck detection methods (e.g., Luikart et al. 1998, Garza & Williamson 2001), especially for populations that had a small pre-bottleneck population size (Williamson-Natesan 2005). This method was implemented using the program BOTTLENECK (Piry et al. 1999), which calculates the heterozygosity expected for each locus under mutationdrift equilibrium  $(H_{eq})$  – given the number of alleles, the sample size, and the specified mutation model – and compares it to the heterozygosity expected under Hardy-Weinberg equilibrium (H<sub>e</sub>). We assumed a variance of 12 repeat units for multi-step mutations [based on the parameterization recommended by Piry et al. (1999)] and a range of biologically-realistic values for the percent of multi-step mutations (0%, 5%, 10%, 15%, and 20%). A one-tailed Wilcoxon

sign ranked test was used to determine if there was evidence of excess heterozygosity ( $H_e > H_{eq}$ ) at a significant number of loci.

We performed the bottleneck tests using a subset of our genetic data because the Cornuet & Luikart (1996) method assumes that: (1) alleles vary by a constant repeat unit, which was not the case for all loci; and (2) the sample is drawn from a panmictic population, which was not the case at the scale of the island (Chapter 3). Therefore, to meet model assumptions, we excluded four loci (see Table 2.1) that did not have a constant repeat unit size (e.g., due to small insertions or deletions). We also restricted our analyses to subregions in the western (n = 66), central (n = 66)145), and eastern (n = 48) portions of the island that had high sample sizes (see Fig. 2.1) and used Mantel tests (Mantel 1967) to confirm panmixia within each of them. To do that, we calculated genetic distances [Rousset's a (Rousset 2000)] and geographic distances (logtransformed) between individuals using SPAGeDi software (Hardy & Vekemans 2002) and performed Mantel tests (Pearson method, 10000 permutations) using the vegan package in R. All three Mantel tests failed to reject the null hypothesis (western subregion: r = 0.01, P = 0.37; central subregion: r = 0.04, P = 0.08; eastern subregion: r = 0.05, P = 0.15), so we proceeded with the bottleneck analyses that assumed panmixia. We also estimated  $N_e$  for each subregion using the approximate Bayesian computation method implemented in the program ONeSAMP (Tallmon *et al.* 2008).

We tested for spatial variation in genetic diversity within the contemporary population of Island Scrub-Jays using the index "homozygozity by loci" (hereafter *HL*; Aparicio *et al.* 2006). This index varies between zero (all loci heterozygous) and one (all loci homozygous) and is calculated using the formula:

$$HL = \frac{\sum E(h)}{\sum E(h) + \sum E(j)}$$
where E(h) is the expected heterozygosity of loci that are *homozygous* in that individual and E(j) is the expected heterozygosity of loci that are *heterozygous* in that individual. This method weights loci based on their allelic diversity and evenness, as a homozygous state results in the greatest increase in *HL* when it occurs at the locus with the highest expected heterozygosity. *HL* values were calculated using the R package *Rhh*, and individuals with missing genotype data were excluded (n = 30).

We tested for spatial autocorrelation in *HL* values using a Mantel test (Mantel 1967), which compared a log-transformed matrix of pairwise geographic distances between individuals to a matrix of pairwise differences in HL values between individuals (Pearson method, 10000 permutations). The test was conducted using the R package vegan. We found evidence for significant spatial autocorrelation (see Results), so next we sought to identify factors responsible for spatial variation in genetic diversity. A priori, we hypothesized that two factors may be involved because of their potential influence on spatial variation in gene flow and effective population size  $(N_e)$ : (1) distance from the center of the island, because the Island Scrub-Jay population on Santa Cruz has limited dispersal and exhibits a pattern of isolation-by-distance (Chapter 3), so habitat patches along the periphery of the island likely get fewer immigrants (Eckert et al. 2008), and (2) percent habitat cover, because Island Scrub-Jays are less abundant when the amount of suitable habitat on the landscape drops below 50% (Sillett *et al.* 2012). We used ArcGIS (ESRI 2011) to calculate the distance each individual was captured relative to the geographic center of Santa Cruz, as well as the proportion of the landscape composed of habitat within 1 km of each capture location (excluding ocean). The location of habitat was determined using a 2005 vegetation map (The Nature Conservancy 2007), and we defined habitat as any vegetation community that included Quercus or Pinus trees. The two explanatory variables were negatively correlated (*Pearson's r* = -0.61, *n* = 533), so we chose to focus our analysis on the distance-to-island-center variable because it should reflect variation in both immigrant potential and jay density (both lower towards the periphery of the island). The distance-to-island-center variable was right-skewed, so a log-transformation was applied. We ran spatial error models (implemented in the R package *spdep*) to test for a relationship between *HL* and distance-to-island-center and assessed the strength of evidence for the relationship using a log-likelihood ratio test (which compared the model to a null model with no explanatory variable). We used spatial error models because they account for non-independence between individuals captured within close proximity (Ward & Gleditsch 2008). We also performed a *post hoc* analysis to test for a relationship between percent habitat cover and the residuals of the distance-to-island center model; the relationship was not significant (log-likelihood ratio test, D = 0.03, P = 0.86, df = 1), indicating that any effect of habitat cover on *HL* was accounted for by the distance-to-island center variable.

Inbreeding depression. We examined evidence for inbreeding depression using data on survival and reproduction, as well as another variable that is less commonly invoked in discussions of inbreeding depression: territory acquisition. We chose to include the latter variable because breeding habitat for Island Scrub-Jays is saturated, so some individuals exist as "floaters" for years until they can acquire a breeding territory [median age of territory acquisition is 4 years (Collins & Corey 1994)]. During the fall field seasons of 2009 to 2011, we captured 145 Island Scrub-Jays on our demography plots that were aged as having hatched that year. Many (n = 102) of those individuals were known to be alive two years after they hatched (based on band-resighting surveys on our demography plots), and we classified those individuals into

two categories: those that acquired a territory on our demography plots (n = 31) and those that did not (n = 71). Individuals that disappeared were excluded, so our territory acquisition variable reflects the probability that an individual that survived the first two years of life acquired a breeding territory in our study area during that time. We tested whether individuals with low genetic diversity (high *HL* values) had a lower probability of territory acquisition using logistic regression (implemented with the glm function in R). In addition to *HL*, we considered hatch year and sex as factors that could also affect an individual's probability of acquiring a breeding territory. We ran models that included all possible combinations (with additive and interaction terms) of *HL*, year, and sex as well as a null model (no explanatory variables), and used Akaike information criterion (AIC) (Burnham & Anderson 2010) to assess the importance of *HL* as a predictor of territory acquisition.

We tested for survival costs of low genetic diversity using a mark-recapture framework (Lebreton *et al.* 1992). Our analysis was restricted to individuals with complete genotype data that were captured within our demography plots from 2009 to 2011 (n = 279). We included data for territorial and non-territorial individuals, even though radio-telemetry work has shown that non-territorial individuals maintain home ranges that extend beyond the study area [encompassing ~8 breeding territories (Mudry 2008)]. Intensive surveys were conducted within the study area to resight banded birds in October and November each year from 2009 to 2013. We chose to use fall (rather than spring) data because non-territorial individuals are easier to observe at that time and because the primary period of natal dispersal is in the summer after individuals hatch (thus, individuals did not enter our resighting dataset until after they dispersed from their natal territory). We treated each month as a capture/resighting occasion and pooled all of the observations from a given month into a single variable that reflected whether or not an

individual was observed. This yielded a dataset with 10 occasions and 1257 observations. We used program MARK (White & Burnham 1999) to fit Cormack-Jolly-Seber models and estimate apparent survival ( $\phi$ ) and recapture probabilities (p). First, we modeled  $\phi$  as a function of territoriality (time-varying individual covariate reflecting whether or not an individual held a territory) and considered a variety of model structures for p. We ran all reasonable combinations of the following factors: resigning occasion (unique p for each occasion), year (unique p for each field season), resighting method (separate p for 20 individuals in fall 2011 that were radiotracked), territoriality, and sex. We determined that the best model structure for p was an additive combination of resighting occasion, territoriality, and sex, so we used that structure for all subsequent models. We then considered four factors hypothesized to drive variation in  $\phi$ : sex, territoriality, winter precipitation (precipitation during or in the winter that preceded the survival interval), and HL. Winter precipitation was calculated as the cumulative amount of precipitation that fell in the Central Valley of Santa Cruz from November to April (data from the Western Regional Climate Center); this method included the vast majority of precipitation because the island is almost always dry from May to October (Schoenherr et al. 2003). We hypothesized that the survival costs of low genetic diversity (if present) would be exacerbated in drier years because winter precipitation is positively correlated with plant productivity and insect abundance on the Channel Islands (Levine et al. 2008; Langin et al. 2009). We ran all possible combinations of the four factors (additive effects, interactions, null model) and evaluated model support using AIC model selection.

We predicted that relatively inbred individuals (i.e., those with high *HL* values) would be less prevalent in the breeding population if they have a lower probability of territory acquisition and/or survival. The above analyses tested for genetic effects on territory acquisition and survival

individually. As an additional test, we also compared the distribution of *HL* values in hatch-year birds (n = 90 males, 55 females) to the distribution in the breeding population (n = 78 males, 68 females), performing separate analyses for males and females. We only included individuals captured on our demography plots and we tested for a significant difference between distributions using a two-tailed Kolmogorov-Smirnov test implemented using R code produced by Holliday (2012). The null hypothesis was that the two samples were drawn from the same continuous distribution.

Finally, we examined evidence for reproductive costs of low genetic diversity using data from nests that were located and regularly monitored each breeding season from 2008 to 2012 [for details see Caldwell et al. (2013)]. Previous work has shown that inbreeding in birds can elevate rates of hatching failure (Briskie & Mackintosh 2004; Marr & Arcese 2006). To test for this in Island Scrub-Jays, we assembled a dataset of nests with known hatching success and complete genotype information, but we lacked sufficient power (n = 47 nests, 33 territories) to test for an effect of genetic diversity and relatedness on hatching success. Therefore, we instead assembled a broader dataset that included all nests with known hatching success (n = 107 nests, 63 territories), and we used those data to estimate the average rate of hatching failure in our study population. We assumed that an egg failed to hatch when the number of nestlings was less than the number of eggs observed in a nest (parents remove unhatched eggs, so it is difficult to differentiate between hatching failure and a partial-predation event). We restricted our analysis to only one nest per female per year; when there were data from two nests, only the first was included. We then calculated the mean hatching failure (percent of eggs in nest that failed to hatch) for each year, followed by the overall mean, and compared those statistics to what has been found in other studies of inbred avian species.

## Results

### Island-mainland comparison

The Island Scrub-Jay had less genetic diversity at neutral loci than its mainland congener (Table 2.2), confirming previous findings that were based on a much smaller sample of Island Scrub-Jays (Delaney & Wayne 2005). At all loci that could be compared (n = 5), Island Scrub-Jays had fewer alleles (*average percent difference* = 56%; paired t-test,  $t_4 = 3.5$ , P = 0.03) and lower levels of heterozygosity (*average percent difference* = 36%; paired t-test,  $t_4 = 3.4$ , P = 0.03) compared to Western Scrub-Jays that had been sampled across mainland California [the clade of Western Scrub-Jays that is a sister lineage to the Island Scrub-Jay (Delaney *et al.* 2008; McCormack *et al.* 2011)].

### Temporal and spatial variation in genetic diversity

There was no evidence that habitat loss and fragmentation over the past 200 years caused a reduction in genetic diversity below historical levels. We tested for genetic signatures of a recent population bottleneck using data from three subregions (see Fig. 2.1), each of which was panmictic, and found that Island Scrub-Jays did not have excess heterozygosity compared to what would be expected under mutation-drift equilibrium (Table 2.3). This result was consistent across subregions, and was also robust to variation in the assumed mutation model.

Spatial factors did influence genetic diversity, however, despite the fact that the species occupies a small geographic range. There was significant spatial autocorrelation in levels of within-individual genetic diversity (Mantel test, r = 0.05, P = 0.003), and Island Scrub-Jays located towards the periphery of the island were more homozygous (higher *HL* values) than

individuals located towards the center of the island (log-likelihood ratio test, D = 16.2, P < 0.0001, df = 1; Fig. 2.2).

### Inbreeding depression

On our demography plots, young jays that were more homozygous had a lower probability of acquiring a breeding territory within the first two years of their life. All of the territory-acquisition models that received support (i.e.,  $w_i > 0$ ) in Table 2.4, except for one, included an effect of *HL* (*cumulative AIC<sub>c</sub> weight* = 0.97) and the confidence interval estimated for the *HL* parameter in the top model (year, sex, *HL*, year\*sex) did not overlap zero ( $\beta$  = -4.93, 95% *CI* = -7.25 to -2.61). Furthermore, the effect size for *HL* in the top model was substantial: an individual that was heterozygous at all of the loci we measured (*HL* = 0) would be predicted to have a 0.5 higher probability of acquiring a territory within the first two years of its life compared to an individual that was relatively inbred (*HL* = 0.55).

In contrast, there was no evidence that individuals with low genetic diversity suffered survival costs, either prior to acquiring a breeding territory or afterwards. *HL* received strong model support as a predictor of apparent survival (*cumulative AIC<sub>c</sub> weight* = 0.98; Table 2.5). However, the direction of the effect was opposite to predicted and did not apply to all individuals. Estimates of  $\phi$  for the top model (territoriality, *HL*, territoriality\**HL*) increased with *HL* in non-territorial individuals and did not vary with *HL* in territorial individuals (Fig. 2.3). We have reason to believe that relatively outbred, non-territorial individuals have lower estimated rates of apparent survival (true survival\*fidelity) because they have a higher rate of territory acquisition outside of our demography plots (i.e., lower fidelity), not because they have a lower rate of true survival (see Discussion).

Despite the effect of genetic diversity on territory acquisition, the distribution of *HL* values in the breeding population was not skewed towards individuals that had high levels of genetic diversity (Fig. 2.4). For both males and females, the Kolmogorov-Smirnov tests failed to reject the null hypothesis that the two *HL* distributions – representing hatch-year birds and the breeding population – were drawn from the same continuous distribution (males: D = 0.1, P = 0.76; females: D = 0.2, P = 0.17).

Hatching failure in Island Scrub-Jays was not unusually high. The annual rate of hatching failure was 9.5%, on average, from 2008 to 2012 (*range* = 2.5% to 15.2%; *n* = 98 nests), which is similar to the typical rate of hatching failure (~10%) for bird populations at the same latitude as Santa Cruz (Koenig 1982) and is lower than rates documented for populations that have undergone bottlenecks (>10%; Briskie & Mackintosh 2004).

### Discussion

Populations that are endemic to islands tend to have lower genetic diversity than related mainland populations (Frankham 2008). This phenomenon could reflect historical factors, as many insular populations were founded by few individuals and have persisted in isolation over long time scales with low  $N_e$ . However, low genetic diversity could also be a product of recent anthropogenic changes (e.g., habitat loss, introduction of novel predators), which are known to have particularly negative impacts on populations that are restricted to islands (Whittaker & Fernandez-Palacios 1999; Ricketts *et al.* 2005; Johnson & Stattersfield 2008). We found that the Island Scrub-Jay had lower levels of neutral genetic diversity than its sister lineage on the mainland of California (Table 2.2), but there was no evidence that habitat loss due to overgrazing in the 19<sup>th</sup> and 20<sup>th</sup> Centuries caused a reduction in genetic diversity below historical levels

(Table 2.3).

Many factors may have contributed to the relatively low historical levels of genetic diversity in the Island Scrub-Jay. Aphelocoma jays have weak flying abilities (Pitelka 1951) and the northern Channel Islands have never been connected to the California mainland (Johnson 1978), so the founding population was almost certainly restricted to a small number of individuals that made it across the Santa Barbara Channel during the Pleistocene when sea level was lower (Collins 2009). After that the population was bounded by island size. The founding population occupied a relatively large (~2000 km<sup>2</sup>) island – Santarosae – but as sea level rose during the Holocene the island was fragmented into four smaller islands, the largest of which are Santa Cruz (250 km<sup>2</sup>) and Santa Rosa (215 km<sup>2</sup>) (Schoenherr et al. 2003). The contemporary population of Island Scrub-Jays has been isolated on Santa Cruz for ~10,000 years, as there is no evidence of on-going gene flow with mainland populations (Delaney & Wayne 2005; McCormack *et al.* 2011), and has persisted despite low  $N_e$  (Table 2.3). The reduced genetic diversity we detected in the Island Scrub-Jay population, therefore, is likely the product of a founder effect during the initial colonization event and chronic genetic drift due to the population's isolation and small size.

Within the contemporary population, additional spatial factors have also acted to reduce the heterozygosity of some individuals. We found that Island Scrub-Jays located towards the periphery of Santa Cruz had higher homozygosity-by-loci values (i.e., lower heterozygosity) than individuals located towards the center of the island (Fig. 2.2). This finding is consistent with previous studies that have detected lower genetic diversity towards the range margin in a range of taxa [e.g., Lammi *et al.* 1999, Lönn & Prentice 2002, Schwartz *et al.* 2003; reviewed by Eckert *et al.* (2008)], but in those cases genetic diversity was lower in peripheral *populations* –

not along the periphery of a single population. Many hypotheses have been posited to explain the observed pattern of lower genetic diversity in peripheral populations (Eckert *et al.* 2008): (1)  $N_e$  may be lower because they occupy marginal habitat, making them more susceptible to genetic drift; (2) they may experience lower levels of gene flow because immigrants can arrive from fewer directions; and (3) they may carry the historical legacy of founder effects associated with extinction/recolonization events, which may be more common in marginal environments. In the case of the Island Scrub-Jay, all of those hypotheses are plausible because the population exhibits a pattern of isolation-by-distance across the landscape (Chapter 3), and jay density (Fig. 2.S1) and  $N_e$  estimates (Table 2.3) are higher at the center of Santa Cruz Island. Thus, along the periphery of the island immigrant potential should be lower, the strength of genetic drift should be greater, and the frequency of localized extinction/recolonization in genetic diversity *among* populations can also be important for generating spatial variation in genetic diversity *within* populations.

There appear to be few fitness costs of low genetic diversity in Island Scrub-Jays. Individuals with low homozygosity-by-loci values (i.e., higher heterozygosity) had a higher probability of acquiring a breeding territory early in life, but the breeding population on the whole was not skewed towards individuals that had low *HL* values (Fig. 2.4), indicating that homozygous individuals do acquire territories eventually. Furthermore, there was no evidence that low levels of genetic diversity negatively impacted survival or fecundity. To the contrary, non-territorial individuals with *high* heterozygosity had lower estimated rates of apparent survival (Fig. 2.3a). We believe this result reflects higher rates of territory acquisition outside of our demography plots, not lower rates of survival, because non-territorial individuals usually

have home ranges that extend beyond the area where resighting surveys occurred (Mudry 2008; T.S. Sillett *et al.*, unpubl. data). If they were to acquire a territory outside of the study area – a possibility that is more likely for heterozygous individuals, given the results in Table 2.4 – then we would be unlikely to detect them during future surveys.

A lower rate of territory acquisition could have a negative impact on the fitness of homozygous individuals, but it is unlikely to be an important factor driving population-level fitness. Florida Scrub-Jays (A. coerulescens) are similar to Island Scrub-Jays in that they are strongly limited by habitat (i.e., breeding vacancies), and long-term research on that species has shown that one of the key determinants of lifetime reproductive success is the number of years an individual is a breeder (Woolfenden & Fitzpatrick 1984). If that is the case for Island Scrub-Jays as well, then more homozygous individuals may suffer negative fitness consequences if they cannot acquire a breeding territory until they are 3+ years old. That is unlikely to affect population-level fitness, however, because it does not affect the number of individuals in the breeding population; that number is limited by habitat availability. It is possible that we lacked sufficient power to detect subtle survival and/or reproductive costs of low genetic diversity. It is also possible that we could not detect costs because inbreeding depression only appears in those demographic variables during especially harsh environmental conditions (half of the years of our study were on the dry end of the spectrum for southern California, but they did not represent maximum drought conditions; see Fig. 2.S2). Nevertheless, our data have positive implications for Island Scrub-Jay conservation because they show that current levels of genetic diversity do not appear to be having a strong, negative impact on the fitness of the only extant population.

These results will be useful for managers who are in the process of developing a longterm conservation strategy for the species (Morrison *et al.* 2011). The Island Scrub-Jay was

recently uplisted to "vulnerable" on the IUCN Red List (IUCN 2012) because the entire species is restricted to one small island, where it is susceptible to short-term threats like fire and the arrival of West Nile Virus. One long-term strategy that has been proposed involves reintroducing Island Scrub-Jays to Santa Rosa Island, which would create an "insurance" population that would make the species less susceptible to catastrophic events (Morrison *et al.* 2011). Inbreeding depression is a common concern when establishing new populations (Jamieson *et al.* 2006), and it is encouraging that few fitness costs were detected in the extant population of Island Scrub-Jays, despite relatively low levels of genetic diversity compared to the mainland. That said, inbreeding depression could appear in the reintroduced population because environmental conditions on Santa Rosa differ from those on Santa Cruz [Santa Rosa is colder, with some differences in vegetation communities (Schoenherr *et al.* 2003)].

Conservation biologists have debated extensively about whether demographic or genetic factors pose a greater threat to the viability of small populations (Lande 1988; Lehman 1998; Jamieson 2007). Our results indicate that the genetic threat of inbreeding depression is not a pressing conservation concern for Island Scrub-Jays, at least compared to more immediate threats like fire and disease. Over longer time scales, however, genetic factors could play a significant role in determining whether Island Scrub-Jays persist on the Channel Islands. Climate change projections for southern California predict that there will be a shift towards a drier climate (Cayan *et al.* 2008), and the Island Scrub-Jay will need to respond to future environmental changes *in situ* – either through adaptive plasticity or through evolutionary changes – because they will not have the option of moving due to limited dispersal abilities. Historical levels of genetic diversity were clearly sufficient to enable the species to survive (1) drastic environmental changes that accompanied the transition from the Pleistocene to the

Holocene, and (2) habitat alterations that were a consequence of ranching activities in the 19<sup>th</sup> and 20<sup>th</sup> Centuries. To ensure the species' resilience is maintained in the future, conservation efforts should focus on guarding against any losses of genetic diversity (e.g., due to fire, disease). This will require protecting Island Scrub-Jays in different regions of the island because the population is not panmictic and instead exhibits spatial variation in genetic structure (Chapter 3). Managers may also want to consider translocation efforts within Santa Cruz to increase densities in peripheral regions of the island, where genetic drift appears to be stronger.

# **Tables and figures**

**Table 2.1** Summary of twelve microsatellite loci used for genetic analyses. Columns show repeat motif (tri = three nucleotides; tetra = four nucleotides), size range (in bp), number of alleles (A), observed heterozygosity (H<sub>0</sub>), expected heterozygosity (H<sub>E</sub>), and the number of individuals genotyped (n). Asterisks denote loci that were excluded from analyses in Table 2.3 because the size differential between alleles was not always consistent (e.g., due to small insertions or deletions).

Locus	Repeat	Size	Α	Ho	HE	n
ApCo2	tri	238-247	4	0.58	0.59	563
ApCo30	tri	187-214	7	0.30	0.31	563
ApCo37	tri*	131-156	6	0.61	0.59	563
AIAAGG13	tetra	185-209	3	0.58	0.54	563
MJG6	tetra*	190-325	34	0.95	0.95	554
PJGATA2	tetra	126-154	7	0.72	0.73	563
PJGATA3	tetra	317-341	7	0.79	0.80	563
PJAAAG9	tetra*	373-424	13	0.81	0.81	559
CmAAAG6	tetra	208-256	13	0.80	0.82	563
CmAAAG11	tetra	220-328	27	0.92	0.94	562
CmAAAG25	tetra	226-314	20	0.91	0.91	554
CmAAAG30	tetra*	441-604	39	0.95	0.96	549

**Table 2.2** A comparison of genetic diversity in Island Scrub-Jays (*A. insularis*) versus Western Scrub-Jays (*A. californica*) at five microsatellite loci. Island Scrub-Jays were sampled across Santa Cruz Island, California, and Western Scrub-Jays were sampled across mainland California [data for *A. californica* are from (Delaney & Wayne 2005)]. Columns give number of alleles (A), observed heterozygosity (H<sub>0</sub>), and sample size (n).

	Island Scrub-Jays			Western Scrub-Jays		
Locus	Α	Ho	n	Α	Ho	n
ApCo2	4	0.58	564	21	0.93	51
ApCo30	7	0.30	564	21	0.93	52
ApCo37	6	0.61	564	14	0.91	54
AIAAGG13	3	0.58	564	29	0.96	53
MJG6	34	0.95	555	37	0.97	42
average	11	0.60	562	24	0.94	50

**Table 2.3** Estimates of effective population size ( $N_e$ ) and results of bottleneck tests for the western (n = 66), central (n = 145), and eastern (n = 48) subregions (locations shown in Fig. 2.1). We tested for evidence of excess heterozygosity (Cornuet & Luikart 1996) assuming a range of biologically-realistic parameterizations for the percent of multi-step mutations (0-20%); p-values for those tests are shown (a recent bottleneck is inferred when P < 0.05).

Subregion	N <sub>e</sub> (95% CI)	Heterozygosity-excess tests				
		0%	5%	10%	15%	20%
western	73 (63-128)	0.42	0.37	0.19	0.16	0.13
central	409 (268-1126)	0.42	0.32	0.13	0.13	0.13
eastern	58 (49-94)	0.42	0.96	0.88	0.81	0.77

**Table 2.4** Model selection results for a logistic regression analysis testing factors hypothesized to influence the probability that an Island Scrub-Jay acquires a breeding territory within the first two years of its life. The factors considered were hatch year (2009, 2010, 2011), sex (male, female), and *HL* (an index of within-individual genetic diversity). Columns give model descriptions, number of parameters estimated (K), second-order Akaike information criterion difference values corrected for small sample size ( $\Delta AIC_c$ ), and  $AIC_c$  weights (w<sub>i</sub>).

Model	K	<b>∆AIC</b> <sub>c</sub>	Wi
year, sex, HL, year*sex	7	0.00	0.57
year, sex, HL, sex*HL	6	2.13	0.20
year, sex, HL, year*sex*HL	12	2.95	0.13
year, HL	4	5.83	0.03
sex, HL, sex*HL	4	6.01	0.03
year, sex, year*sex	6	7.17	0.02
year, sex, HL	5	7.69	0.01
year, HL, year*HL	6	9.53	0.00
HL	2	10.63	0.00
year	3	10.83	0.00
year, sex, HL, year*HL	7	11.50	0.00
sex, HL	3	12.63	0.00
year, sex	4	12.82	0.00
null (no predictors)	1	16.25	0.00
sex	2	18.25	0.00

**Table 2.5** Model selection results for a mark-recapture analysis testing factors hypothesized to influence apparent survival ( $\phi$ ) in Island Scrub-Jays. The factors considered were sex (male, female), territoriality (time-varying individual covariate reflecting whether or not an individual held a territory), precipitation (precipitation during or in the winter that preceded the survival interval), and *HL* (an index of within-individual genetic diversity). Columns give model descriptions, number of parameters estimated (K), second-order Akaike information criterion difference values corrected for small sample size ( $\Delta AIC_c$ ), and  $AIC_c$  weights (w<sub>i</sub>). Only the top 15 models are shown, accounting for 89% of the  $AIC_c$  weight (results for full model set available upon request). See Figure 2.3 for parameter estimates from the top model.

Model	K	ΔAIC <sub>c</sub>	Wi
territoriality, HL, territoriality*HL	15	0.00	0.21
territoriality, HL	14	0.68	0.15
territoriality, HL, precipitation, territoriality*HL	16	1.61	0.09
territoriality, HL, sex, territoriality*HL	16	1.94	0.08
territoriality, precipitation, HL	15	2.00	0.08
territoriality, sex, HL	15	2.65	0.06
territoriality, HL, sex, precipitation, territoriality*HL	17	3.60	0.03
territoriality, precipitation, HL, precipitation*HL	16	3.95	0.03
territoriality, precipitation, HL, territoriality*precipitation	16	3.97	0.03
territoriality, sex, precipitation, HL	16	4.03	0.03
territoriality, sex, precipitation, HL, sex*precipitation	17	4.14	0.03
territoriality, sex, HL, territoriality*sex	16	4.22	0.03
territoriality, sex, HL, sex*HL	16	4.56	0.02
territoriality, sex, precipitation, HL, territoriality*sex*precipitation	18	4.90	0.02
territoriality, precipitation, HL, territoriality*precipitation*HL	18	5.01	0.02



**Figure 2.1** Locations where genetic samples were collected from Island Scrub-Jays on Santa Cruz Island. The inset shows the location of the island within California, USA. The size of each blue circle is proportional to the number of samples collected within 500 m of the circle's centroid, and the black ovals outline the western, central, and eastern subregions (used for analyses in Table 2.3). The shaded baselayer depicts the topographic relief (layer from ESRI) and is colored maroon in areas that have Island Scrub-Jay habitat.



**Figure 2.2** Within-individual homozygosity (homozygosity by loci, *HL*) in Island Scrub-Jays increased with distance from the center of the species' range on Santa Cruz Island, California. Data are means  $\pm$  95% confidence intervals for individuals grouped into the following distance categories: 0-3 km (n = 185), 3-6 km (n = 184), 6-9 km (n = 109), 9-12 km (n = 33), and 12-15 km (n = 27).



**Figure 2.3** Estimates of monthly rates of apparent survival ( $\phi$ ) for different values of withinindividual homozygosity (homozygosity by loci, *HL*) in Island Scrub-Jays that (a) did and (b) did not hold a breeding territory. Dotted lines depict the 95% confidence intervals around the estimates. The maximum *HL* value for non-territorial individuals was 0.55 and the maximum value for territorial individuals was 0.44 (see Fig. 2.4).



**Figure 2.4** Distribution of homozygosity-by-loci values in Island Scrub-Jays that (a) were part of the non-territorial "floater" population in the year they hatched (n = 145), and (b) acquired breeding territories at some point in their life (n = 146). All birds were located on the demography plots where we tested for evidence of inbreeding depression. There was no evidence that the breeding population was skewed towards individuals that were more heterozygous. Males and females were analyzed separately (see text), but they did not differ so were combined here for visualization purposes.

# Supplementary tables and figures

Multiplex	Loci (and 5'-	Q-solution	PCR cycling conditions
•	fluorescent labels)	added?*	• 0
1	AIAAGG13 (VIC)	Ν	5 min. at 95°C; 30 cycles of 30 sec. at
	ApCo2 (PET)		94°C, 90 sec. at 56°C, and 30 sec. at
	MJG6 (NED)		72°C; 30 min. at 60°C
2	ApCo30 (6-FAM)	Y	5 min. at 95°C; 30 cycles of 30 sec. at
	ApCo37 (VIC)		94°C, 90 sec. at 52°C, and 30 sec. at
			72°C; 30 min. at 60°C
3	PJGATA3 (VIC)	Ν	5 min. at 95°C; 30 cycles of 30 sec. at
	PJAAAG9 (NED)		94°C, 90 sec. at 56°C, and 30 sec. at
			72°C; 30 min. at 60°C
4	PJGATA2 (6-FAM)	Y	5 min. at 95°C; 30 cycles of 30 sec. at
	CmAAAG6 (PET)		94°C, 90 sec. at 56°C, and 90 sec. at
	CmAAAG11 (VIC)		72°C; 30 min. at 60°C
	CmAAAG25 (NED)		
	CmAAAG30 (6-FAM)		

 Table 2.S1
 Summary of the multiplex PCR conditions.

\*Q-solution is an optional reagent in QIAGEN's type-it microsatellite PCR kit.



**Figure 2.S1** The predicted density of Island Scrub-Jays in 9 ha grid cells (n = 2787) in relation to the cells' distance from the center of Santa Cruz Island. Sillett *et al.* (2012) generated these estimates using point-count data that were collected in October 2008 at 369 randomly distributed locations across the island, and a hierarchical distance-sampling model that included habitat and elevation as predictor variables. Density estimates were significantly higher towards the center of the island (log-likelihood ratio test comparing autoregressive lag models that did and did not include distance-to-island-center as a predictor variable, D = 59.8, P < 0.0001, df = 1; analyses were performed on log-transformed density data using the *spdep* package in R).



**Figure 2.S2** The demographic data used to test for inbreeding depression were collected in years that represent much of the range of variation in winter precipitation, a climate parameter that has been shown to influence the demography of bird populations in southern California (Morrison & Bolger 2002; Langin *et al.* 2009; Sofaer *et al.* 2012). Open circles represent years for which demographic data were collected on Island Scrub-Jays (n = 6 years), and closed circles represent earlier years (n = 59 years from 1941 to 2007; data were missing for some years). These data represent winter (November to April) precipitation in Santa Barbara, California (data obtained from the Western Regional Climate Center), which is on the mainland adjacent to Santa Cruz Island.

CHAPTER 3. Islands within an island: repeated adaptive divergence within a single population<sup>2</sup>

### Summary

Adaptive divergence within populations is thought to be rare due to constraints imposed by gene flow and recombination. Yet, the capacity for a single population to diversify and adapt to multiple ecological opportunities is a fundamental assumption of theory explaining resource polymorphisms, sympatric speciation, and adaptive radiations. Some models provide a resolution to this dilemma by outlining conditions that allow for adaptive divergence within populations. The prevalence of these conditions in nature, however, remains unknown. Here we document genetic structure and spatially-replicated patterns of adaptive divergence within a bird species that consists of a single population endemic to Santa Cruz Island, California. Island Scrub-Jays (Aphelocoma insularis) in three separate stands of pine habitat had longer, narrower bills than genetically-related jays in adjacent oak habitat, a pattern that mirrors adaptive differences between allopatric populations of the species' mainland congener. We also found that Island Scrub-Jays mated non-randomly with respect to bill morphology and that gene flow was reduced between pine and oak habitats. Many organisms are exposed to fine-scale environmental heterogeneity and exhibit localized dispersal, suggesting that adaptive divergence within populations may be more common than is currently appreciated.

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# Main text

A long-standing debate in evolutionary biology has centered on the relative importance of geography and ecology as factors generating biological diversity. Geographic isolation of populations has traditionally been viewed as the primary scenario favoring evolutionary divergence (Mayr 1963; Via 2001; Bolnick & Fitzpatrick 2007). However, there is growing appreciation for the role of ecological variation and natural selection in driving divergence, even in the absence of geographic barriers (Feder et al. 1988; Rundle & Nosil 2005; Schluter 2009). Theoretical models demonstrate that multiple lineages can evolve within a single population when strong divergent selection is coupled with non-random mating based on the trait under selection (Maynard Smith 1966; Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Bolnick 2006). Yet, while these models provide support for the plausibility of divergence in sympatry, debate has continued over how common this process is in nature because of the paucity of well-supported empirical examples (Via 2001; Bolnick & Fitzpatrick 2007). A major impediment to resolving this debate has been the difficulty of reconstructing circumstances under which past divergence events have occurred (Grant & Grant 2010). An alternative approach is to identify populations in the initial stages of sympatric divergence and investigate whether they fall within the range of conditions described in theoretical models (Bolnick & Fitzpatrick 2007). We carry out such a test here in a species that consists of a single, closed population exposed to spatially-replicated divergent selective pressures.

The Island Scrub-Jay (*Aphelocoma insularis*) is a non-migratory, passerine bird restricted to Santa Cruz Island, a 250 km<sup>2</sup> island in southern California, USA. At first glance, this species appears to be an unlikely candidate for tests of fine-scale adaptive divergence because it has small census and effective population sizes (Delaney & Wayne 2005; Sillett *et al.* 2012), lacks

physical barriers to dispersal within its geographic range, and is an ecological generalist (Curry & Delaney 2002). Indeed, the most reasonable *null* expectation is that Island Scrub-Jays represent one homogenous population devoid of phenotypic or genetic structure. Other aspects of this system, however, make it well-suited for testing whether divergent selection pressures have led to sympatric divergence. First, no evidence exists for either multiple colonization events or ongoing hybridization with the mainland sister species, the Western Scrub-Jay (A. californica; Delaney & Wayne 2005; McCormack et al. 2011). We can therefore preclude any effects of gene flow from outside the population. Second, research on allopatric populations of Western Scrub-Jays across western North America provides directional predictions for patterns of adaptive divergence across the mosaic of habitats on Santa Cruz Island. Coastal populations of A. californica occupying oak (Quercus spp.) habitat have shorter, deeper bills than inland populations occupying pine (Pinus spp.) habitat (Peterson 1993). This pattern has been attributed to divergent selection driven by variation in the availability of different seed crops, as long, narrow bills are more efficient for feeding on pine cones while short, deep bills are more efficient for feeding on acorns (Bardwell et al. 2001). Most Island Scrub-Jays inhabit oak woodland and chaparral, but a subset of the population occurs in three spatially-separated stands of Bishop Pine (P. muricata; Fig. 3.1b) – all of which are within the potential dispersal radius of jays in oak habitat (Fig. 3.S1) – providing an opportunity to test for adaptive divergence in sympatry.

We tested for divergence in bill morphology and genetic structure within the population by capturing, measuring, and sampling 563 Island Scrub-Jays across Santa Cruz Island from 2009 to 2011 (see Supplementary Methods for details). This intensive effort sampled ~25% of the species' entire population size (Sillett *et al.* 2012) and included individuals from all three

pine stands (Fig. 3.S2). We found that the size and shape of Island Scrub-Jay bills, but not their overall body size, varied consistently between habitats: individuals captured in pine habitat (n =112) had longer, narrower bills than those captured in oak habitat (n = 351; Tables 3.S1-S2; Figs. 3.1, 3.S3-S5). This pattern was repeated in all three pine stands (Fig. 3.1c) and mirrors bill differences that have been documented in allopatric populations of Western Scrub-Jays occupying pine and oak habitat at a continental scale (Peterson 1993). Bill length and depth were both heritable, based on an analysis of related individuals (see Supplementary Discussion). We also found that Island Scrub-Jays in oak habitat had more "pine-like" bills when they were closer to pine habitat (log-likelihood ratio test on bill length: T = 18.6, P < 0.001, df = 1; bill depth: T =0.23, P > 0.05, df = 1; Fig. 3.2), a pattern suggesting gene flow between habitats, and one that is opposite to predictions if oak and pine habitats induced two discrete bill types via phenotypic plasticity or if long-billed birds were simply choosing pine habitats (see Supplementary Discussion for more detailed rationale for ruling out phenotypic plasticity and other alternate explanations). Thus, the spatial pattern in bill morphology of Island Scrub-Jays is consistent with a scenario of adaptive evolutionary divergence across a heterogeneous landscape.

To test if this adaptive divergence was associated with a reduction in gene flow between habitat types, we genotyped all individuals at 12 microsatellite loci and examined spatial patterns in neutral genetic divergence. Our analyses rejected the null hypothesis of panmixia: interindividual genetic distance increased with geographic distance across Santa Cruz Island (Mantel test, r = 0.11, P < 0.0001; Fig. 3.S6), and a spatial principal components analysis (Jombart *et al.* 2008) detected positive autocorrelation in allele frequencies (global permutation test, P = 0.01). A subsequent landscape genetics analysis indicated that both ecological and geographic factors contributed to the observed spatial genetic patterns because the top model, which included habitat type (oak vs. pine) and longitude (distance), was 30 times more likely than a model with distance alone (Table 3.S3). The habitat effect was due to a genetic discontinuity associated with the boundary between the largest pine stand and adjacent oak habitat (Fig. 3.3a; Table 3.S4), which was present despite the absence of any geographic barrier. The remainder of the spatial genetic structure was due to isolation-by-distance across the island's 38 km east-west axis. These genetic results show that Island Scrub-Jays in all three pine stands were more closely related to individuals in adjacent oak habitat than they were to those in other pine stands (see panels a-c in Fig. 3.3). Remarkably, each pine stand can therefore be considered an independent case where adaptive divergence has been maintained in the face of some gene flow with adjacent oak birds.

Most models of sympatric divergence incorporate a mechanism that restricts mating between adaptively-divergent ecotypes (Maynard Smith 1966; Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Bolnick 2006). In support of those models, our genetic analysis detected a reduction in gene flow between oak and pine habitat (Fig. 3.3a), which could be due to a link between bill morphology and mate choice (Servedio *et al.* 2011), natural selection against dispersing individuals (Nosil *et al.* 2005), and/or habitat selection based on natal origin (Davis & Stamps 2004). We also found evidence that Island Scrub-Jays mate nonrandomly with respect to bill morphology: long-billed females were more likely to pair with long-billed males ( $r^2 = 0.35$ , P < 0.0001, n = 43; Fig. 3.4), even when the analysis was restricted to oak habitat ( $r^2 = 0.24$ , P = 0.003, n = 34). This result may in part be due to active mate selection based on bill morphology (Huber *et al.* 2007). However, an alternative explanation is that non-random mating is simply a by-product of the combined effect of limited dispersal across the landscape (i.e., isolation-by-distance; Fig. 3.3) and spatial variation in bill morphology generated by divergent selection (Fig. 3.2). In other words, long-billed females may have a

higher likelihood of mating with long-billed males because that is the most common bill morphology in their local neighborhood.

Collectively, our results demonstrate how divergent selection can generate repeated patterns of adaptive divergence within populations that exhibit limited dispersal and non-random mating across a heterogeneous landscape. Island Scrub-Jays have been evolving in isolation for approximately one million years (McCormack et al. 2011), so the morphological differences between pine and oak birds may represent locally-adapted ecotypes, rather than a case of nascent speciation (Bolnick 2006). Our data nevertheless provide empirical support for models that invoke divergent selection and non-random mating as drivers of sympatric speciation (Maynard Smith 1966; Kondrashov & Kondrashov 1999; Bolnick 2006). They are also congruent with the recent perspective that localized dispersal (i.e., isolation-by-distance) may be an important mechanism underlying patterns of non-random mating within populations that are exposed to spatially-divergent selection (Mallet et al. 2009). Localized dispersal and fine-scale environmental heterogeneity are common properties of natural populations and landscapes (Sexton et al. 2014). Therefore, similar patterns of within-population divergence may be more widespread than is currently appreciated, but have gone undocumented because studies are rarely designed to test for adaptive divergence at the scale of individual habitat patches – particularly in mobile taxa. More empirical research is needed on populations exposed to fine-scale divergent selection to understand the range of conditions that constrain and facilitate the evolution of adaptive differences. Discovering and conserving such cryptic diversity may also be important for maintaining the adaptive potential of range-restricted species like the Island Scrub-Jay, which often have low genetic diversity and cannot disperse to track environmental changes.

## **Tables and figures**



**Figure 3.1** Island Scrub-Jays in all three of Santa Cruz Island's pine stands had bills that were longer and narrower than jays in oak habitat. (a) Standardized bill photograph, showing an Island Scrub-Jay from oak habitat biting on a dowel, with a size standard affixed to its lower mandible. (b) Map of Santa Cruz Island, with colors representing habitat type (orange = oak, green = pine) and labels referring to the western, central, and eastern pine stands (see Fig. 3.3c for a magnified view of the small eastern pine stand). (c) The effect size (model-averaged  $\beta$ -values  $\pm$  95% confidence intervals) for differences in bill length (closed circles) and bill depth (open circles) in the three pine stands compared to oak habitat (horizontal dotted line). The analyses were performed using spatial error models that tested for morphological differences between individuals captured in pine habitat (west *n* = 57; central *n* = 22; east *n* = 33) and oak habitat (*n* = 351), while controlling for sex and age.



**Figure 3.2** Island Scrub-Jays had shorter bills when they were located further away from pine habitat. The groups are adult males (closed circles), adult females (open circles), young males (closed diamonds), and young females (open diamonds), the data are means  $\pm$  95% confidence intervals for individuals in each distance category, and the numbers are sample sizes. The maximum distance an individual was captured away from pine habitat was 6.1 km. The difference in bill length between pine jays and jays in oak >4 km distant from pine habitat is of a similar magnitude as the difference documented for allopatric populations of Western Scrub-Jays occurring in pine and oak habitat (Peterson 1993).



**Figure 3.3** The Island Scrub-Jay population exhibited spatial genetic structuring across Santa Cruz Island, California, USA. Regions outlined in black represent pine habitat and the remaining colored regions represent oak habitat. The color gradient is a continuous representation of the predicted genetic surface for the first axis of a spatial principal component analysis (sPCA1), based on parameter estimates from the top model of an analysis that tested for factors contributing to variation in sPCA1 (Tables 3.S3-S4). The analysis used genotype data from 563 individuals captured across the island (representing ~25% of the total population). We detected an east-west gradient and a genetic discontinuity at the boundary of the largest, western pine stand (but not the smaller, central and eastern pine stands) that was equivalent to genetic variation across 3 km of longitude. The magnitude of genetic differentiation is such that the probability of allelic identity between an individuals. Insets a-c show the western, central, and eastern pine stands and adjacent oak habitat (all to the same scale). Inset d shows Santa Cruz relative to the other Channel Islands and mainland California.



**Figure 3.4** Island Scrub-Jays mated non-randomly with respect to bill length. Each point represents a pair of breeding birds (n = 43), and the line gives the best linear fit to the data. All individuals were measured as adults. The same result was obtained when controlling for body size.

### **Supplementary methods**

#### Study system

Island Scrub-Jays are restricted to Santa Cruz Island, California, USA, and have a total population size of fewer than 3000 individuals (Sillett et al. 2012). Genetic diversity is low relative to the species' mainland congener (Chapter 2) and phylogenetic analyses indicate that Island Scrub-Jays diverged from mainland *Aphelocoma* approximately one million years ago (McCormack et al. 2011). During that time, Santa Cruz Island has experienced marked changes in vegetation communities. Coniferous forests predominated during glacial intervals in the Pleistocene, while oak was more widespread during warmer interglacials (Anderson et al. 2009). Oak (primarily Island Scrub Oak, Q. pacifica) forms the majority of woody vegetation across the contemporary landscape and is fragmented because of topographic variation and past effects of livestock overgrazing during the 19<sup>th</sup> and 20<sup>th</sup> Centuries (Junak 1995). Bishop Pines also survive as climatic relicts in areas that receive considerable inputs of marine fog (Fischer et al. 2009) and occur in distinct stands intermixed with oak in the western (418 ha), central (224 ha), and eastern (21 ha) portions of the island [note that a fire reduced the extent of the eastern pine stand in the 1990s (Walter & Taha 1999)]. Island Scrub-Jays typically occur in one habitat type or the other because they have extremely small territories [ $3.5 \pm 0.2$  ha (mean  $\pm$  SE), n = 129 (Caldwell *et al.* 2013)] that they maintain year-round. Intense competition for space forces some individuals to exist as "floaters" until they can acquire a breeding territory (Collins & Corey 1994), but they still maintain home ranges that encompass ~8 breeding territories (Mudry 2008).

Island Scrub-Jays have a generalist diet similar to mainland *Aphelocoma* (Pitelka 1951; Curry & Delaney 2002) and consume a variety of items ranging from plant matter to insects to small vertebrates. Acorns are a key component of their diet, especially during the non-breeding
season (Curry & Delaney 2002). Individuals in pine habitat also forage on *P. muricata* cones, which are serotinous and open in response to hot temperatures (natural fires on Santa Cruz Island are rare; Walter & Taha 1999). Island Scrub-Jays often inspect open cones while foraging, and we have observed them obtaining invertebrates (e.g., spiders) from pine cones in addition to pine seeds. Individuals in pine habitat also frequently collect, cache, and feed on acorns.

# Field sampling

We sampled 563 Island Scrub-Jays from 2009 to 2011 at widely-distributed locations (see Fig. 3.S2), with a maximum distance between sampled individuals of 29 km. A helicopter was used to access some of the remote sites due to the rugged terrain on the island and the limited road network. Most individuals were captured with baited drop-traps or mist-nets. Each individual was marked with a unique combination of numbered aluminum and colored leg bands and aged as after-hatch-year (referred to as adults) and hatch-year (referred to as young birds) according to plumage differences described by Pyle (1997). Measurements and photographs were then taken for morphological analyses (see below). Blood (20-60 µL) was collected from the brachial vein of all captured jays and stored in lysis buffer (0.1M Tris HCl, 0.1M EDTA, 0.01M NaCl, 0.5% SDS) until DNA extraction. Genetic samples of nineteen jays in remote areas were acquired from feathers found on the ground; if individuals had also been captured in the same region, feather and blood genotypes were later compared to eliminate duplicate sampling. Longitude and latitude were recorded with GPS receivers at every sampling location.

For each captured jay, the following measurements were recorded with digital calipers (to  $\pm 0.01$  mm): bill length, measured from the anterior end of the nares to the tip of the bill; bill depth, measured at the anterior end of the nares; and tarsus length. Wing (unflattened) and tail

length were also measured with a ruler (to  $\pm 0.5$  cm). To eliminate observer error, KML measured all of the Island Scrub-Jays included in the morphological analyses. The bills of 332 individuals were measured three times per capture event to reduce measurement error and to estimate repeatability (Lessells & Boag 1987), which was high for both bill measurements: length (r = 0.99) and depth (r = 0.94). The majority (n = 463) of Island Scrub-Jays were captured between late August to early December to minimize seasonal effects; data from jays captured during other seasons were excluded from morphological analyses unless otherwise noted.

We also took standardized digital photographs of 423 of the 463 jays captured during the fall to quantify aspects of bill morphology that could not be measured with calipers. The photographs were taken with a Canon Powershot SX10 IS as the birds were biting on a dowel placed perpendicular to the camera; the camera and the dowel were both secured to a metal stand at a distance of 40 cm from one another, and a size standard was affixed to the bird's lower mandible (Fig. 3.1a). The photos were used for morphometric analyses (see below).

# Behavioral data

We collected data on territorial and reproductive behavior from a subset of Island Scrub-Jays, mostly focused on individuals living within three study plots in oak habitat (Caldwell *et al.* 2013). We determined breeding pairs of Island Scrub-Jays through a combination of behavioral observations and nest monitoring. KML measured both the male and the female (when they were adults) for 43 pairs to test for non-random pairing by bill morphology. We also documented 22 natal dispersal events (i.e., situations where the natal origin was known for territorial adults), 18 of which were for males and 4 of which were for females. Natal origin was determined for breeding birds using two methods: (1) the individual had been given a numbered aluminum leg

band as a nestling (n = 13), and (2) the individual had been captured during their first fall and their genotypic profile (see below) was used to assign parentage to breeding pairs (at a 95% confidence level) using CERVUS software (Kalinowski *et al.* 2007; n = 9). We calculated natal dispersal distance by measuring the distance from the center of each bird's natal territory to the center of their first breeding territory.

# Microsatellite genotyping

The methods followed those detailed for microsatellite genotyping in Chapter 2. Briefly, DNA was extracted from blood and feather samples using a DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA). All samples were genotyped at 12 variable microsatellite loci (see Table 2.1) using QIAGEN's type-it microsatellite PCR kit and a Vapo-Protect Mastercycler 6321 (Eppendorf, Hauppange, NY, USA). PCR products were run on an Applied BioSystems 3730xl DNA Analyzer, and the resulting electropherograms were scored automatically and checked manually using GeneMarker (SoftGenetics, State College, PA, USA). Negative and positive controls were run with each batch of reactions to check for contamination and repeatability, respectively. The entire process from amplification to allele scoring was also repeated for 8% of the samples (n = 46) at all 12 loci, and we detected no discrepancies.

### Molecular sexing

We performed molecular sex identification on birds included in the morphological analyses. Although males tend to be larger than females, the only method to definitively sex Island Scrub-Jays in the field is through behavioral observations (breeding behavior, sex-specific vocalizations), which were not possible for all individuals. We used the 2550F/2718R primer set

and a slightly modified version of the PCR protocol described in Fridolfsson & Ellegren (1999). The PCR products were run on a 2% agarose gel with TBE as the running buffer, visualized under UV light, and scored blind with respect to the identity of the sample. The error rate for this method was 1% (2 of 210 known-sex birds were sexed incorrectly, both of which were female), a low error rate compared to other avian studies that used molecular sexing techniques (Robertson & Gemmell 2006).

## Morphological analyses

We used spatially-explicit linear models (Ward & Gleditsch 2008) to identify factors that explained variation in bill length and depth and – for comparative purposes – body size. Body size was summarized using the first axis generated from a principal components analysis (PCA) on tarsus, wing, and tail length, all of which loaded positively onto the axis. Both bill metrics were positively associated with body size (Pearson correlation coefficients: bill length, r = 0.36; bill depth, r = 0.39) and with each other (r = 0.52). Model predictors were: sex, to control for known gender differences in overall body size; age, to account for the possibility that young birds had not completed growth by the time they were captured; and habitat type, to test for morphological differences between pine and oak habitat.

To assign habitat type, we reclassified a 2005 vegetation map of Santa Cruz Island (The Nature Conservancy 2007) into three categories: (1) oak-dominated chaparral and oak woodland (hereafter referred to as oak habitat), (2) mixed Bishop Pine-oak woodland (hereafter referred to as pine habitat), and (3) all other vegetation types (representing habitat unsuitable for Island Scrub-Jays; see Fig. 3.S2). We used ArcGIS software (ESRI 2011) to calculate the distance from each capture location to the nearest pine polygon. Individuals were categorized as being located

in pine habitat if they were captured within 300 m of pine [the diameter of the largest Island Scrub-Jay breeding territories (Caldwell *et al.* 2013)].

We analyzed the morphological data with spatial error models of the form:

$$y = \beta_0 + \beta_1 x + \varepsilon + \lambda W \xi$$

where  $\varepsilon$  and  $\xi$  represent the non-spatially-autocorrelated and spatially-autocorrelated portions of the error term, respectively,  $\lambda$  reflects the degree of spatial autocorrelation in the error term, and W is a spatial weights matrix (in our case inverse-distance weights between pairs of individuals). This method accounts for non-independence between individuals captured within close proximity, and was performed using the *spdep* package in R (R Core Team 2012). Three parameters ( $\beta$  values) were estimated for habitat type that reflected whether individuals were captured within 300 m of the western, central, and eastern pine stands, whereas for sex and age only one parameter was estimated reflecting whether individuals were male or female and adults or young birds, respectively. Models were run separately on the three response variables and each model set consisted of 15 models, which included a null model (no predictors) as well as all possible combinations of habitat, sex, and age (with additive and interaction terms). AIC model selection (Burnham & Anderson 2010) was used to assess the relative importance of the three model predictors. We examined the model output to detect "pretending" variables (Anderson 2007) and excluded models with interaction terms when calculating model-averaged parameter estimates for the main effects.

We ran additional models to test for the presence of spatial patterns within oak habitat when we found evidence that a morphological variable differed between habitats. Our expectation was that individuals closer to pine habitat would have morphology more similar to "pine individuals." Birds captured in pine habitat were excluded from these analyses, and two

spatial error models were run with the remaining individuals: a model that only included sex and age effects (simple model), and a model that included sex, age, and distance to the nearest pine stand (general model). Because the scale of the distance-to-pine variable was too large relative to  $\lambda$ , causing model instability, we applied a linear transformation to rescale it from 0 to 1. Likelihood ratio tests were used to assess the strength of evidence for the general model.

We also performed a geometric morphometric analysis (Rohlf & Marcus 1993) on the bill photographs to quantify habitat-related variation in bill shape while controlling for size differences between individuals. We used TPS software (Rohlf 2010) to place homologous landmarks at the tip of the bill and immediately above and below the anterior end of the nares perpendicular to the mandibular tomium, as well as sliding semi-landmarks along the outline of the upper mandible (see Fig. 3.S3). The observer placing the landmarks was blind to the capture location of the individual (e.g., oak vs. pine). For a subset of captures (n = 28), duplicate photographs were taken (after the birds were removed and returned to the photograph apparatus) and the landmark data from those photographs were used to assess the repeatability of landmark placement. We calculated the area enclosed by the landmarks (measured using the splancs package in R) and found high repeatability (r = 0.93). After excluding duplicate photographs, we used TPS software to slide semi-landmarks (Bookstein 1991) and align the specimens, and used MorphoJ software (Klingenberg 2011) to perform a Procrustes fit and generate covariance matrices. This method removed non-shape related variation in morphology by rotating the coordinates and scaling them relative to centroid size (a measure of size based on the square root of the sum of the squared distances of each landmark to the centroid). Canonical variate analyses (CVAs) were then used to describe the features of shape that varied between individuals in pine and oak habitat, and permutation tests (with 10000 iterations) were conducted to determine if

those differences were significant. Sex and age were found to contribute to variation in bill shape, so separate CVAs were performed on each combination of sex and age.

Finally, we used an animal model approach (Kruuk 2004; Wilson et al. 2010) to determine if variation in our two linear bill metrics, length and depth, was heritable. A pedigree was constructed using known offspring-parent relationships for Island Scrub-Jays located within three study plots in oak habitat [see map in Caldwell et al. (2013)]. Genetic parentage was confirmed using CERVUS software (at a 95% confidence level) because Island Scrub-Jays engage in extra-pair copulations (Delaney 2003). Individuals were included in the analysis when we had measurement data for the offspring and at least one parent (n = 27 offspring from 13 families). We estimated the proportion of phenotypic variance explained by the pedigree -i.e., narrow-sense heritability  $(h^2)$  – using the *MCMCglmm* package in R, which uses Bayesian inference. We ran 500,000 Markov chain Monte Carlo iterations, with a 200,000 iteration burnin and a thinning of 500. Sex and age were included as fixed effects in the models. The results we report (see Supplemental Discussion) are based on a flat prior (equal probability assigned to each variance component, which equates to  $h^2 = 0.5$ ) with a low degree of belief (nu = 1), a parameterization we decided upon *a priori*. However, we also ran models with different priors  $(h^2 = 0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 0.9)$  and found that  $h^2$  estimates varied from 0.44 to 0.75  $(0.56 \pm 0.04 \text{ (mean} \pm \text{SE}), n = 9)$  for bill length and 0.10 to 0.59  $(0.38 \pm 0.05, n = 9)$  for bill depth. The exact value of our reported estimates should therefore be interpreted with caution because our estimates were sensitive to the choice of prior. Nevertheless, we found strong evidence that variation in bill morphology in Island Scrub-Jays has a heritable component.

# Genetic analyses

To assess spatial genetic structure, we first used a Mantel test (Mantel 1967). This statistic computes correlations between dissimilarity matrices and has been widely applied in landscape genetics research to test for an association between genetic and geographic distance (Manel *et al.* 2003). We used the *vegan* package in R to perform the Mantel test (Pearson method, 10000 permutations) on a log-transformed matrix of pairwise geographic distances between individuals and a matrix of pairwise genetic distances between individuals [using Rousset's *a* (Rousset 2000)]. We used SPAGeDi software (Hardy & Vekemans 2002) to calculate Rousset's *a*, an index of the degree of genetic divergence between two individuals based on the probability of allelic identity within versus between *individuals* (rather than *populations*, as  $F_{ST}$  is used for).

The second method we employed to test for spatial genetic structure was a spatial principal components analysis (sPCA; Jombart *et al.* 2008). This approach is similar to a PCA in that it takes a multivariate dataset (e.g., microsatellite genotypes) and produces a reduced number of orthogonal axes. However, it also incorporates spatial information by partitioning the product of variance in allele frequencies and the degree of spatial autocorrelation in allele frequencies (using Moran's I). This produces axes that describe spatial patterns of genetic variation. Some axes reflect positive spatial autocorrelation, referred to as global structures, which could be due to isolation-by-distance and/or barriers; others reflect negative spatial autocorrelation, referred to as local structures, which could reflect repulsion between genetically similar individuals across the landscape. The network structure used to estimate spatial autocorrelation was based on inverse-distance weights between pairs of individuals. We used the *adegenet* package in R to

perform a spatial principal components analysis and to conduct permutation tests (with 10000 iterations) on the resulting axes to test for the presence of both global and local genetic structure.

Both the Mantel test and the spatial principal components analysis rejected the null hypothesis of panmixia. We therefore performed an additional landscape genetics analysis to identify factors responsible for spatial genetic structuring across the geographic range of the Island Scrub-Jay. Because partial Mantel tests have been criticized recently (Guillot & Rousset 2013), we modeled the sPCA output using the same spatial-error model structure as described in the morphological analysis section. We used scores for the first sPCA axis as the response variable because there was evidence for global structure and the largest discontinuity between eigenvalues was between the first and second sPCA axes, which is the recommended method for determining which axes to retain for further analyses.

Model predictors were: latitude, longitude, habitat type, and habitat fragmentation. Linear transformations were applied to the latitude and longitude data, resulting in values ranging from 0 to 1. These transformed factors were used to represent the hypothesis that spatial genetic structure in Island Scrub-Jays represents a continuous pattern of genetic variation across the island, driven by limited dispersal distance (i.e., a pattern of isolation-by-distance). Habitat type and fragmentation categories were used to represent two different hypotheses regarding factors that may cause genetic discontinuities across the landscape. The first hypothesis represented an ecological barrier (habitat type), and the second represented a physical barrier (habitat fragmentation). Habitat type was treated the same as described in the morphological analyses, and we estimated three parameters to reflect whether individuals were captured within 300 m of the western, central, and eastern pine stands. Habitat fragmentation, in contrast, was modeled by grouping individuals into patches of contiguous habitat (regardless of habitat type). Island Scrub-

Jays prefer habitat that represents a mosaic of woody vegetation and open areas (Sillett *et al.* 2012; Caldwell *et al.* 2013). We therefore classified habitat fragmentation based on two different thresholds – 20% and 40% – and used ArcGIS to create polygons representing areas where the amount of woody vegetation on the landscape did not drop below the threshold amount (using moving windows with a 300 m radius). Individuals were assigned to the nearest polygon, resulting in 9 different groupings using the 20% fragmentation threshold and 15 different groupings using the 40% threshold. Full models (latitude, longitude, habitat type, habitat fragmentation) were run for each habitat-threshold value. We found more support for the 20% fragmentation threshold and used those polygon assignments in all analyses. Maximum likelihood was computed for all additive combinations of the four model predictors (latitude, longitude, habitat type, and habitat fragmentation), and AIC model selection was used to assess which predictors contributed to spatial genetic structure (i.e., variation in sPCA1 scores).

### **Supplementary discussion**

# Alternative explanations for differences in bill morphology

Island Scrub-Jays in all three of the island's pine stands had longer, narrower bills, a pattern consistent with predictions based on a hypothesis of trophic adaptation as documented in Western Scrub-Jays (Peterson 1993; Bardwell *et al.* 2001). Three alternative explanations for this pattern are possible, however: phenotypic plasticity, morphology-driven habitat selection, and adaptation to different thermal environments. Below, we evaluate each of these alternative explanations.

Bill morphology has a well-documented genetic basis, as it has been linked to a number of genes and quantitative trait loci (Abzhanov *et al.* 2006; Knief *et al.* 2012) and it has been

shown to be heritable in many bird species (Smith & Zach 1979; Boag 1983). Our animal model analysis indicated that bill morphology has a heritable component in Island Scrub-Jays as well [h<sup>2</sup> estimates (and 95% credible intervals) for bill length: 0.55 (0.21-0.84); and bill depth: 0.42 (0.08-0.73)]. However, some of the residual phenotypic variance could reflect plasticity due to (1) variation in the outer layer of bird bills, which is composed of keratin and can vary in thickness due to growth and wear (Matthysen 1989), or (2) variation in conditions during development (Gil et al. 2008). These explanations are unlikely to account for habitat-related differences in bill morphology in A. insularis for the following reasons. Bill length in Island Scrub-Jays does vary seasonally, but seasonal variation is consistent across habitats (Fig. 3.S7). We therefore have no evidence that bill wear is habitat-dependent (e.g., due to prying open pine cones). Furthermore, neither mode of plasticity can account for the strong relationship between bill length and distance-to-pine within oak birds (Fig. 3.2). Phenotypic plasticity due to variation in growth or wear would be predicted to generate a sharp change in bill morphology across the pine-oak habitat boundary and no spatial pattern within habitat types (see Fig. 3.S8a). Developmental plasticity would be expected to produce a pattern with intermediate phenotypes near the oak-pine boundary, because some individuals may have dispersed between habitat types, but no spatial pattern beyond the zone of potential dispersal between pine and oak habitats (see Fig. 3.S8b). We found that bill morphology in Island Scrub-Jays changed gradually in relation to distance from pine habitat, a pattern that extended beyond the species' potential dispersal distance, at least for males (Fig. 3.2). Hence, although we have evidence of phenotypic plasticity in bill morphology (Fig. 3.S7), neither of the two modes of plasticity can account for the pattern of spatial variation in bill morphology in oak birds (Fig. 3.2).

Another potential explanation for the patterns we observed involves morphology-driven habitat selection (Edelaar et al. 2008). Island Scrub-Jays might preferentially settle in a particular habitat according to their bill morphology, with longer-billed jays selecting pine habitat and shorter-billed jays selecting oak habitat. This hypothesis requires that Island Scrub-Jays have the opportunity to select between the two habitat types, which appears to be a rare event for males in particular. We know the natal origin for 22 individuals that became territorial adults on the three study plots where we have been monitoring jay reproductive ecology since 2008 (Caldwell et al. 2013). All of the male dispersal events we detected (n = 18) have been less than a kilometer (Fig. 3.S1), typically within 2-3 territories of their natal territory. This cannot be attributed to a limited ability to detect longer dispersal events because all of the females that we detected (n = 4)established breeding territories at distances greater than 2 km from their natal territory, consistent with female-biased dispersal that has been previously documented in Florida Scrub-Jays [A. coerulescens (Coulon et al. 2010)] and many other bird species (Pusey 1987). These data suggest that most male Island Scrub-Jays that hatch in oak habitat do not have the option of selecting pine habitat (see Fig. 3.S2). Furthermore, the morphology-driven habitat selection hypothesis predicts that the greatest habitat-related difference in male bill morphology should occur within 1 km of the transition between pine and oak habitat (i.e., within the spatial scale at which habitatbased sorting could occur; see Fig. 3.S8c), but we found the reverse: Island Scrub-Jays had shorter bills the further they were from pine habitat (Fig. 3.2).

Finally, spatial variation in jay bill morphology could have evolved because of temperature gradients, not habitat heterogeneity. Recent studies have shown that bills can serve as organs for the conductance of dry heat (Tattersall *et al.* 2009; Greenberg *et al.* 2012a) and have found that larger bills – due to their greater surface area for heat transfer – may be at a

selective advantage in hot environments (Symonds & Tattersall 2010; Greenberg *et al.* 2012b). For example, Greenberg and Danner (Greenberg & Danner 2012) found that Song Sparrows (*Melospiza melodia*) in California had larger bills in warmer, drier locations. Ambient temperature on Santa Cruz Island varies spatially due to a combination of coastal influence and topography (Junak 1995). Bishop Pines are restricted to relatively cool, high elevation locations that receive consistent moisture input from marine fog (Fischer *et al.* 2009). The thermaladaptation hypothesis, therefore, predicts that larger bills would be at a selective advantage in the hotter, drier oak habitat. We found that Island Scrub-Jay bills were longer in pine habitat (Fig. 3.1), with a minimal difference in surface area (pine birds had bills with 2% greater surface area, on average; see Supplementary Methods for details on surface area calculation using landmark data). This difference in bill surface area was both opposite to the direction predicted and much lower than what has been found in studies supporting the thermal-adaptation hypothesis (e.g., Greenberg *et al.* (2012a) reported a 17% difference in bill surface area between *M. melodia* subspecies that occupy different thermal environments).

# Supplementary tables and figures

**Table 3.S1** Candidate set of spatial error models to test hypothesized predictors of variation in (a) bill length, (b) bill depth, and (c) body size (first axis of PCA on tarsus, wing, and tail length). Columns give model descriptions, number of parameters estimated (K), second-order Akaike information criterion difference values corrected for small sample size ( $\Delta AIC_c$ ),  $AIC_c$  weights (w<sub>i</sub>), and estimates of the spatial autocorrelation parameter ( $\lambda$ ). The cumulative  $AIC_c$  weight ( $\Sigma w_i$ ) for each model predictor is also shown at the bottom.

Model	K	ΔAIC <sub>c</sub>	Wi	λ
sex, age, habitat, sex * age	9	0.0	0.62	0.12
sex, age, habitat	8	2.4	0.19	0.12
sex, age, habitat, age * habitat	11	2.9	0.15	0.12
sex, age, habitat, sex * habitat	11	5.7	0.04	0.12
sex, age, habitat, sex * age * habitat	18	9.3	0.01	0.12
sex, age, sex * age	6	31.3	0.00	0.15
sex, age	5	33.5	0.00	0.15
sex, habitat	7	56.8	0.00	0.11
sex+ habitat, sex * habitat	10	60.9	0.00	0.11
sex	4	87.6	0.00	0.13
age, habitat	7	330.0	0.00	0.02
age, habitat, age * habitat	10	332.5	0.00	0.02
age	4	350.9	0.00	0.06
habitat	6	361.0	0.00	0.01
null (no predictors)	3	381.9	0.00	0.05

a) Bill length:

 $\Sigma w_i$ : sex = 1.00, age = 1.00, habitat = 1.00

b) Bill depth:

Model	K	ΔAIC <sub>c</sub>	Wi	λ
sex, age, habitat	8	0.0	0.53	0.12
sex, age, habitat, sex * age	9	1.3	0.27	0.12
sex, age, habitat, age * habitat	11	3.1	0.11	0.12
sex, age	5	5.4	0.04	0.12
sex, age, habitat, sex * habitat	11	5.8	0.03	0.12
sex, age, sex * age	6	6.7	0.02	0.12
sex, age, habitat, sex * age * habitat	18	14.6	0.00	0.12
sex, habitat	7	38.7	0.00	0.12
sex	4	42.1	0.00	0.13
sex, habitat, sex * habitat	10	44.8	0.00	0.12
age, habitat	7	310.8	0.00	0.05
age	4	312.1	0.00	0.06
age, habitat, age * habitat	10	313.9	0.00	0.05
habitat	6	335.6	0.00	0.05
null (no predictors)	3	336.2	0.00	0.06

 $\Sigma w_i$ : sex = 1.00, age = 1.00, habitat = 0.95

c) Body size:

Model	K	ΔAIC <sub>c</sub>	Wi	λ
sex, age, habitat, sex * age	9	0.0	0.55	0.07
sex, age, sex * age	6	0.5	0.44	0.09
sex, age, habitat	8	9.7	0.00	0.08
sex, age, habitat, sex * age * habitat	18	10.1	0.00	0.08
sex, age	5	10.1	0.00	0.09
sex, age, habitat, age * habitat	11	11.0	0.00	0.08
sex, age, habitat, sex * habitat	11	14.5	0.00	0.08
sex	4	287.2	0.00	0.10
sex, habitat	7	292.1	0.00	0.10
sex, habitat, sex * habitat	10	298.0	0.00	0.10
age	4	538.1	0.00	-0.08
age, habitat	7	538.6	0.00	-0.10
age, habitat, age * habitat	10	541.8	0.00	-0.10
null (no predictors)	3	658.8	0.00	-0.04
habitat	6	663.4	0.00	-0.04

 $\Sigma w_i$ : sex = 1.00, age = 1.00, habitat = 0.56

**Table 3.S2** Model-averaged parameter estimates (and 95% confidence intervals) from the candidate set of spatial error models that tested hypothesized predictors of morphological variation in Island Scrub-Jays. Positive  $\beta$ -values for sex, age, and pines indicate that males, adults, and pine birds, respectively, had larger morphological measurements.

Trait	D	Model-averaged		
Iran	Parameter	β-value		
bill length	sex	1.95 (1.86 to 2.03)		
	age	0.67 (0.59 to 0.76)		
	western pines	0.64 (0.49 to 0.79)		
	central pines	0.85 (0.62 to 1.07)		
	eastern pines	0.83 (0.63 to 1.03)		
bill depth	sex	0.52 (0.50 to 0.55)		
	age	0.16 (0.14 to 0.18)		
	western pines	-0.07 (-0.11 to -0.04)		
	central pines	-0.16 (-0.22 to -0.10)		
	eastern pines	-0.10 (-0.16 to -0.05)		
body size	sex	2.34 (2.27 to 2.41)		
	age	1.40 (1.33 to 1.47)		
	western pines	-0.04 (-0.11 to 0.02)		
	central pines	-0.01 (-0.11 to 0.08)		
	eastern pines	0.21 (0.13 to 0.29)		

**Table 3.S3** Candidate set of spatial error models to test hypothesized predictors of spatial genetic structure in Island Scrub-Jays (using the first spatial principal component axis as the response variable). Columns give model descriptions, number of parameters estimated (K), second-order Akaike information criterion difference values corrected for small sample size  $(\Delta AIC_c)$ , AIC<sub>c</sub> weights (w<sub>i</sub>), and estimates of the spatial autocorrelation parameter ( $\lambda$ ). The cumulative AIC<sub>c</sub> weight ( $\Sigma w_i$ ) for each model predictor is also shown at the bottom. \*Note that latitude is a "pretending variable" [i.e., is uninformative (Anderson 2007)] because its addition to the top model only changes the  $\Delta AIC_c$  value by ~2, which is the penalty for the addition of one parameter.

Model	K	<b>∆AIC</b> <sub>c</sub>	Wi	λ
longitude, habitat type	7	0.0	0.68	0.05
longitude, latitude*, habitat type	8	1.9	0.27	0.05
longitude	4	6.7	0.02	0.05
longitude, latitude	5	8.8	0.01	0.05
longitude, fragmentation	12	8.8	0.01	0.04
longitude, habitat type, fragmentation	15	9.8	0.01	0.04
longitude, latitude, fragmentation	13	9.9	0.00	0.04
longitude, latitude, habitat type, fragmentation	16	10.1	0.00	0.05
latitude, habitat type, fragmentation	15	48.7	0.00	0.09
latitude, fragmentation	12	63.8	0.00	0.09
habitat type, fragmentation	14	75.9	0.00	0.08
fragmentation	11	85.1	0.00	0.09
latitude, habitat type	7	174.8	0.00	0.12
habitat type	6	181.4	0.00	0.12
latitude	4	220.8	0.00	0.14
null (no predictors)	3	229.3	0.00	0.14

 $\Sigma w_i$ : latitude = 0.01, longitude = 1.00, habitat type = 0.95, fragmentation = 0.02

**Table 3.S4**  $\beta$ -values (and 95% confidence intervals) estimated for parameters in the top model of the landscape genetics analysis (Table 3.S3). Note that the effect size for the  $\beta$ -value associated with the western pine stand is equivalent to genetic variation across 3 km of longitude; see the map in Fig. 3.3 for a visual representation of these parameter estimates.

Parameter	β-value
longitude	1.54 (1.44 to 1.64)
western pines	-0.16 (-0.20 to -0.11)
central pines	0.06 (-0.01 to 0.13)
eastern pines	-0.03 (-0.10 to 0.03)



**Figure 3.S1** All of the pine habitat on Santa Cruz Island is within the potential dispersal radius of Island Scrub-Jays in oak habitat, consistent with sympatry as per Mayr's definition (Mayr 1947). (a) Histogram showing the proportion of pine habitat within 100-m-distance-intervals of oak habitat (green bars). (b) Dot plot showing natal dispersal distances for males (blue dots; n = 18) and females (red dots; n = 4) for whom the locations of both the natal and breeding territories were known. All of the natal dispersal events were detected within and between three study plots in oak habitat from 2008 to 2013. The sex difference in natal dispersal distance is consistent with female-biased dispersal documented in the congener *A. coerulescens* (Coulon *et al.* 2010) and in many other bird species (Pusey 1987).



**Figure 3.S2** Locations where Island Scrub-Jays were sampled across Santa Cruz Island (n = 563). The sizes of the dots correspond to the number of individuals sampled in that area; individuals within 1 km of each other were pooled for visualization purposes. The island is colored according to habitat type (orange = oak woodland and chaparral; green = mixed pine-oak woodland; white = unsuitable habitat for Island Scrub-Jays).



**Figure 3.S3** Standardized bill photograph, which shows an Island Scrub-Jay, with a size standard affixed to its lower mandible, biting on a dowel. Photos were used for a landmark-based geometric morphometric analysis of bill shape. Circles show the landmarks that were placed along the upper mandible; orange circles represent homologous landmarks and white circles represent sliding landmarks.



**Figure 3.S4** The shape of Island Scrub-Jay bills differed significantly between pine and oak habitats in all age/sex groups (P < 0.0001 for all permutation tests). The figures show canonical variate (CV) scores describing habitat-related variation in bill shape for (a) adult males, (b) young males, (c) adult females, and (d) young females. Boxes represent the inter-quartile range (IQR), whiskers extend to 1.5 times the IQR, and circles are outliers. Sample sizes are below each box. Separate CV analyses were performed for each age/sex group.



**Figure 3.S5** Bill shapes represented by the canonical variate (CV) scores in the geometric morphometric analysis (see Fig. 3.S4). Shapes outlined by the dark green and light orange dots (positioned at landmark locations) represent those associated with the maximum and minimum CV scores, respectively, for the CV analyses on (a) adult males, (b) young males, (c) adult females, and (d) young females. These shape analyses controlled for variation in centroid size, which is largely driven by variation in bill length; therefore, differences in bill length are not apparent in these images and differences in bill depth are amplified (relative to raw measurement data). Island Scrub-Jays captured in pine habitat had more positive CV scores than jays captured in oak habitat. These images show that more positive CV scores were associated with higher bill length-to-depth ratios, and not with variation in other components of shape, like curvature.



**Figure 3.S6** A comparison of pairwise genetic distance [Rousset's *a* (Rousset 2000)] across six geographic distance categories: 0-5 km (n = 72801), 5-10 km (n = 51168), 10-15 km (n = 24183), 15-20 km (n = 7792), 20-25 km (n = 2018), and 25-30 km (n = 241). Error bars represent 95% confidence intervals.



**Figure 3.S7** Seasonal variation in bill length was consistent across pine (closed circles) and oak (open circles) habitats (general linear model with individual as a random effect: season effect, P = 0.007; season\*habitat interaction, P = 0.88). Data are means  $\pm 95\%$  confidence intervals for adult Island Scrub-Jays that were captured in fall (late August – early December) and recaptured in spring (June) in oak (n = 19) and pine (n = 7) habitat. Bill depth did not vary seasonally in either habitat (season effect, P = 0.84; season\*habitat interaction, P = 0.79; data not shown). Bill morphology can change due to seasonal variation in the growth and wear of the rhampotheca (the outer keratin layer of the bill; Matthysen 1989). We suspect that seasonal changes in bill length in Island Scrub-Jays reflect growth during the summer molting period, when keratin is being produced, followed by wear during the fall and winter months, when jays feed on nuts that may abrade their bill. Regardless of the mechanism, these data show that seasonal bill variation is of a similar magnitude in oak and pine habitat, so phenotypic plasticity due to variation in bill wear cannot explain habitat differences in bill length.



Figure 3.S8 Predicted spatial patterns in bill length for territorial male Island Scrub-Jays, based on the hypothesis that differences between pine (dark green) and oak (light orange) habitats are driven by phenotypic plasticity (a and b) or morphology-driven habitat selection (c). The predictions are based on the <1 km mean dispersal distance documented for male Island Scrub-Jays (see Fig. 3.S1) and the extremely small home range estimates for territorial individuals [3.5  $\pm 0.2$  ha (mean  $\pm$  SE), n = 129 (Caldwell *et al.* 2013)]. They would be different for females, which disperse further, and for young individuals, which have larger home ranges. (a) Phenotypic plasticity due to variation in foraging, preening, etc. predicts a sharp change in bill morphology across the pine-oak habitat boundary and no spatial pattern within habitat types. (b) Phenotypic plasticity due to variation in conditions during development predicts intermediate phenotypes near the oak-pine boundary, because some individuals may have dispersed between habitat types, but no spatial pattern beyond the zone of potential dispersal between pine and oak habitats. (c) Morphology-driven habitat selection predicts the largest disparity in bill morphology between habitat types should occur within the dispersal radius of pine habitat (the area within which Island Scrub-Jays have the option of choosing between habitats). None of these predictions are consistent with the observed spatial pattern in bill morphology shown in Fig. 3.2.

CHAPTER 4. Bill morphology is linked to acoustic signal structure in a single-island endemic bird: implications for microgeographic divergence<sup>3</sup>

# Summary

Adaptive evolutionary divergence is most likely to occur within populations when divergent natural selection acts on traits that also influence mate choice (so-called "magic" trait hypothesis). Bill size in some bird species appears to function as a "magic" trait because strong selection for foraging on specific food resources causes correlated changes in trilled, male songs that are important for mate choice. Here we test for a relationship between bill morphology and the structure of a broad-band, female-specific call produced by the Island Scrub-Jay (Aphelocoma insularis), a species that is restricted to one small, ecologically-heterogenous island (Santa Cruz Island, California, USA). We previously found adaptive divergence in bill morphology across habitat gradients: birds in pine habitat had longer, narrower bills than birds in adjacent oak habitat. Here we demonstrate that bill morphology is also related to the structure of the female "rattle" call, which is associated with female-male interactions. Females with longer bills produced calls that were more rapid, and those with deeper bills produced calls that were higher in frequency. We found no relationship between vocal signal structure and habitat characteristics, indicating that this pattern cannot be attributed to acoustic adaptations for optimizing sound transmission. Instead, our results suggest that bill morphology may be acting as a "magic" trait in the Island Scrub-Jay. Further research is needed to understand the relationship between bill morphology and vocal signal structure in Island Scrub-Jays, and to

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determine how this linkage contributes to non-random mating within the population. Nevertheless, these findings provide empirical support for mechanisms that have been proposed to facilitate adaptive evolutionary divergence within populations exposed to divergent selection pressures.

# Introduction

The evolution of mating signals has played a central role in driving the diversification of animal taxa (West-Eberhard 1983). Divergence in acoustic, visual, and behavioral traits that influence mating success can act as a premating isolation mechanism and facilitate speciation through the evolution of reproductive isolation (Lande 1981; Servedio 2004; Ritchie 2007). For example, mating signals are widely recognized as being important during the reinforcement phase of allopatric speciation (Coyne & Orr 2004). However, mating signals may also be an important factor underlying adaptive evolutionary divergence within populations when physical barriers to gene flow are lacking (Bolnick & Fitzpatrick 2007; Servedio & Kopp 2012; Richardson *et al.* 2014).

A critical requirement for evolutionary divergence in sympatry is non-random mating between individuals experiencing divergent selection (Dieckmann & Doebeli 1999). In theory, recombination and gene flow should hinder the generation of non-random mating by preventing the build-up of linkage disequilibrium between traits under natural selection and those related to mate choice (Mayr 1963). Therefore, divergence in sympatry is most likely to occur when (1) the ecological trait under natural selection contributes directly to mate choice ("magic" trait), or (2) the ecological trait is genetically linked to a trait related to mate choice ("non-magic" trait complex; Servedio & Kopp 2012). Despite skepticism regarding the biological plausibility of

these scenarios (Gavrilets 2004), an increasing number of studies are finding support for the "magic trait" hypothesis (Servedio *et al.* 2011). This body of theory was developed in the context of sympatric speciation, but is also relevant to understanding how adaptive evolutionary divergence can arise at microgeographic scales within populations subject to disruptive or divergent selection pressures (Bolnick 2006; Richardson *et al.* 2014).

Bird bills provide a classic example of a trait that can be subject to divergent selection and can also contribute to non-random mating (Podos 2001; Servedio *et al.* 2011). The bills of Darwin's finches (*Geospiza* sp.), for example, are adapted to foraging on specific food resources (Boag & Grant 1981; Grant & Grant 2011). At the same time, bill morphology is correlated with the structure of species-specific songs (Podos 2001; Huber & Podos 2006). Podos (2001) attributes this finding to the fact that gape width can modify the resonance properties of the vocal tract (Westneat *et al.* 1993). Larger-billed birds cannot move their bill as quickly to alter gape width (Hoese *et al.* 2000), a constraint that places an upper limit on the pace and frequency bandwidth of vocalizations that involve frequency modulations within individual notes (e.g., trilled songs; Podos 1997). Since Podos (2001) first described this pattern, other studies on trilled songs in New World sparrows (Ballentine 2006; Derryberry 2009), finches (Badyaev *et al.* 2008), and woodcreepers (Derryberry *et al.* 2012) have found evidence that the bill may be acting as a "magic" trait in those species as well. Here we test that hypothesis in a single-island endemic passerine, the Island Scrub-Jay (*Aphelocoma insularis*).

*Aphelocoma* jays are a model system for studying adaptive evolutionary divergence because they exhibit pronounced intra- and inter-specific variation in niche space (Rice *et al.* 2003; McCormack *et al.* 2010), social and mating systems (Woolfenden & Fitzpatrick 1984; Burt & Peterson 1993; Berg *et al.* 2011), and morphology (Pitelka 1951; Peterson 1993;

McCormack & Smith 2008). Niche space is a trait that has been particularly labile within the *Aphelocoma* clade (Rice *et al.* 2003), and in many cases morphological changes have accompanied transitions between habitat types (Peterson 1993; McCormack & Smith 2008). For instance, populations of Western Scrub-Jay (*A. californica*) that occur in pine habitat across the interior of North America have longer, straighter bills than populations in oak habitat along the Pacific coast (Peterson 1993). These morphological differences are adaptive because long, narrow bills are more efficient for feeding on pine cones and short, stout bills are more efficient for feeding on acorns (Bardwell *et al.* 2001).

In a previous study (Chapter 3), we documented a parallel pattern of morphological adaptation within the most range-restricted Aphelocoma, the Island Scrub-Jay. The species only occurs on 250 km<sup>2</sup> Santa Cruz Island in southern California, USA, where transitions between oak and pine habitat occur repeatedly at microgeographic scales (Fig. 4.1). Island Scrub-Jays that occur in pine habitat have longer, narrower bills than jays in oak habitat. However, morphology does not change abruptly across the pine-oak boundary. Instead, bill length declines gradually with distance from pine habitat, consistent with a scenario of divergence-with-gene flow. Furthermore, genetic data indicate that individuals in pine habitat are more closely related to individuals in adjacent oak habitat than to individuals in other pine stands, indicating that each pine stand can be considered an independent case where adaptive divergence has been maintained in the face of some gene flow with adjacent oak birds. At the scale of the island, long-billed males are more likely to form breeding pairs with long-billed females. Localized dispersal and spatial autocorrelation in bill morphology may account for some of this pattern of non-random mating within the population, although active mate selection (e.g., based on acoustic signals) could be another contributing factor.

The primary objective of our study was to assess the relationship between bill morphology and vocal signal structure, and thus to test the hypothesis that the bill may be acting as a "magic" trait in Island Scrub-Jays. The acoustic signal on which we focused was the femalespecific rattle call, which is a rapid, broad-band vocalization that is thought to be important for male-female interactions in *Aphelocoma* and other corvids (Goodwin 1976; Woolfenden & Fitzpatrick 1996; Curry & Delaney 2002). We tested for a relationship between the structure of rattle calls (frequency, rapidity) and variation in bill morphology (bill length, bill depth). While doing so, we controlled for body size because syrinx size can constrain the production of acoustic signals (Wallschläger 1980; Laiolo & Rolando 2003).

We also considered two alternate hypotheses regarding factors that could influence variation in the vocal signal structure of female Island Scrub-Jays: cultural evolution and acoustic adaptation. The first posits that geographic variation in songs and calls produced by oscine passerines, which learn vocalizations from tutors, is due to limited dispersal and the vagaries of cultural transmission (Slater 1986, 1989; Podos & Warren 2007). Island Scrub-Jays exhibit a continuous pattern of isolation-by-distance across the east-west axis of the island, and have not been observed to disperse more than 3.5 km from their natal territory (Chapter 3). Variation in acoustic signals with longitude would thus imply cultural evolution. The acoustic adaptation hypothesis, in contrast, asserts that vocal signals are adapted for optimal transmission in a given environment (Morton 1975, Boncoraglio & Saino 2007). Sounds degrade (i.e., change in structure) as they are transmitted from sender to receiver, and vocalizations that have lower frequencies and longer inter-note intervals are expected to experience the least amount of degradation in densely-vegetated habitat (Wiley & Richards 1978). The density of woody vegetation is heterogeneous across Santa Cruz Island (e.g., Fig. 4.S1) and differences in leaf and

branch structure between oak vs. pine habitat could also shape the acoustic environment. Hence, habitat-specific variation in female vocalizations would suggest acoustic adaptation in Island Scrub-Jays. Support for this second hypothesis would indicate that multifarious selection may be acting across habitat gradients to generate morphological adaptations for foraging and acoustic adaptations for optimal sound transmission (Slabbekoorn & Smith 2002b), necessitating tests for both a habitat effect and an effect of bill morphology.

### Methods

### Study system

Corvids produce a wide variety of vocalizations, but they differ from most other oscine passerines in that males do not produce a typical advertisement song (Goodwin 1976). Instead, the vocalization that appears to be most closely associated with pair behavior is the rattle call, which is a rapid, broad-band vocalization that females give in the presence of their mate in association with a visual display (Goodwin 1976; Woolfenden & Fitzpatrick 1996). Many corvids have a species-specific rattle call, leading to speculation that this vocalization is homologous within the Corvidae (Goodwin 1976). Our decision to focus on a female-specific vocalization is unconventional given that most work on avian acoustic signaling has been conducted on male advertisement songs (e.g., Catchpole 1987, Podos & Warren 2007). However, we believe our choice is justified for two reasons. First, we have reason to suspect that mate selection in Island Scrub-Jays does not follow the conventional female-choice model (Catchpole 1987) because (1) breeding is habitat limited, so both sexes compete fiercely for territory vacancies (Collins & Corey 1994), and (2) during the pair-formation process females feed males, but we have never observed the reverse (authors' pers. obs.). Second, the rattle call is a visually-

elaborate, stereotyped vocalization that is thought to be important for territorial defense and interactions between members of a breeding pair in *Aphelocoma* jays (Woolfenden & Fitzpatrick 1996; Curry & Delaney 2002). Females only give rattle calls in response to behaviors conducted by their mate (e.g., fighting territory intruders), and when they do they point their bill upward and bob their body up and down (Curry & Delaney 2002).

## **Recordings**

We recorded rattles produced by territorial females (n = 75) in a range of locations and habitat types (see Fig. 4.1) using a Sennheiser ME67 directional microphone and a Marantz PMD661 digital recorder (digitized with a 48k sample rate). All vocalizations were recorded from September to December 2011 during clear weather (no rain or fog); occasionally recordings were made during breezy conditions (Beaufort wind force scale > 1). We were able to record during the fall months, even though the birds were not breeding, because Island Scrub-Jays maintain pair bonds and defend territories year-round (Curry & Delaney 2002). Most females were individually identifiable because they had a unique combination of colored and aluminum leg bands (n = 48); the remainder were unbanded, but they could be distinguished from one another because of their mate's leg bands (n = 18) or because of differences in territory location (n = 9). Females produce rattles most frequently during territorial disputes. Thus, to facilitate recording, disputes were encouraged by feeding peanuts to neighboring jays to coax them onto the focal female's territory. Nearly all of the recorded rattles were produced when the female's mate was (1) chasing a territory intruder, (2) entering her vicinity (within 10 m), or (3) flying away with a peanut. Supplemental feeding may have altered the structure of the rattle calls, although the metrics we measured (see below) did not differ when comparing rattles between the

first two stimuli and the third (rapidity:  $F_{1,53} = 1.5$ , P = 0.23; center frequency:  $F_{1,25} = 0.6$ , P = 0.44).

## Vocalization measurements and analyses

We used Program Audacity (Audacity Team 2013) to remove low-frequency background noise (by applying a high pass filter set at 400 Hz) and to normalize the amplitude of all rattle calls to 0 db. Two spectrograms were then generated for each rattle call using Program Raven version 1.4 (Bioacoustics\_Research\_Program 2011), one for temporal measurements (Hann window type, 256 window size, 2.67 ms time resolution) and one for frequency measurements (Hann window type, 512 window size, 98.3 Hz frequency resolution). For each rattle call, we selected ten consecutive notes that had the least amount of overlap with other background noise (e.g., vocalizations from other Island Scrub-Jays, heterospecifics) and we used those notes to measure the following variables: (1) rattle rapidity, defined as the average number of notes produced per second, and (2) center frequency, defined as the frequency that separates the sound energy into two frequency intervals of equal energy (see Fig. 4.2 for spectrogram example). Frequency data were not collected if the rattle notes overlapped with vocalizations produced by other birds. We also measured the maximum amplitude of the ten consecutive rattle notes and the maximum amplitude of a representative section of background noise immediately prior to or following the rattle call (most background noise was caused by wind). Frequency data were excluded from analyses if the background noise was >10% as loud as the rattle call.

We calculated the repeatability of our vocalization measurements according to the method outlined in Lessells & Boag (1987). Duplicate measurements were collected for 20 rattle calls at least 7 days after the original measurements were collected, and the repeatability of those

measurements was high for rattle rapidity (r = 0.85) and center frequency (r = 0.95). We also collected data for multiple rattle calls produced by the same female (rattle rapidity: n = 44 females, with an average of 3 recordings per female; center frequency: n = 18 females, with an average of 3 recordings per female), and we used those data to estimate a composite measure of repeatability that reflected measurement repeatability and within-female repeatability. This composite measure was 0.60 for rattle rapidity and 0.51 for center frequency, indicating that more than half of the variance in those variables was accounted for by differences between individuals. These values are in the midrange of repeatability estimates that have been documented for behavioral traits in other studies (Boake 1989; Bell *et al.* 2009). We did not consider inter-quartile range, i.e., the frequency range that encompasses the middle 50% of the sound energy, because repeatability was low (r = 0.12). Average measurement data were used for all subsequent analyses in cases where multiple rattle calls were recorded for the same female.

We treated rattle rapidity (measured in notes per second, Hz) and center frequency (measured in sound waves per second, Hz) as independent variables because they were not correlated (Fig. 4.S2). Visual inspection of the scatterplot for the two variables (Fig. 4.S2) also revealed no indication of an "upper-bound" limit to vocalization performance (Podos 1997), which could occur if females that produce fast rattles have an upper constraint on their frequency range that differs from females that produce slow rattles. For both vocalization variables, we used three general linear models and program JMP (SAS Institute 2013) to test our primary hypotheses, specifically that the structure of rattle calls vary: (1) with variation in bill morphology in accordance with the "magic" trait hypothesis, (2) along the east-west axis of the island because of cultural evolution due to localized dispersal, and (3) with variation in
vegetation structure and habitat type (oak vs. pine) because of local adaptation to the acoustic environment. We confirmed that the data met the assumptions of general linear models. Further details about the bill morphology and habitat analyses are detailed below.

Bill length, bill depth, and tarsus length (a standard metric of body size in birds) were measured on 34 (of 75) females captured during the fall from 2007 to 2011. KML measured all of the individuals according to the methods outlined in Chapter 3. Six individuals were measured in the fall after they hatched but their vocalizations were recorded in later years. In Island Scrub-Jays, bill length varies between hatch-year and after-hatch-year birds captured in the fall (paired t-test,  $t_{14} = -4.3$ , P < 0.0001, n = 15), but the other morphological variables do not (bill depth:  $t_{14}$ = -0.5, P = 0.59, n = 15; tarsus length:  $t_{14} = -0.7$ , P = 0.49, n = 15). We therefore applied a correction to the bill length data (0.77 mm, representing the average difference between age groups in the paired analysis) for birds that were captured in their first year. The morphological variables were not correlated (Table 4.S1) so we included all of them in models testing for an effect of morphology on vocalization structure (i.e., on rattle rapidity and, in a separate model, center frequency). We detected an east-west trend in rattle rapidity for the entire dataset (see Results), but not for the subset represented by the 34 measured females ( $F_{1,34} = 1.2$ , P = 0.28, n =35). Because longitude was moderately correlated with bill depth (Table 4.S1), we did not include longitude as a covariate in the model testing for an effect of morphology on rattle rapidity (unlike the habitat model; see below).

Habitat variables were generated using LiDAR data that were collected in 2010 (Channel Islands National Park, unpubl. data) and a vegetation map produced in 2005 (The Nature Conservancy 2007) for Santa Cruz Island. To produce metrics of vegetation structure, we used ArcGIS (ESRI 2011) to calculate the following variables within 100 m buffers around each focal female's location [the average radius of Island Scrub-Jay territories (Caldwell *et al.* 2013)]: (1) mean vegetation height (range: 1.2 to 5.1 m, n = 74) and (2) the proportion of Island Scrub-Jay habitat covered by vegetation > 0.5 m in height (range: 0.01 to 0.93, n = 74). We only included vegetation communities that had oak and pine trees and excluded man-made structures, grasslands, rocky slopes, and other open and non-native habitat types. One female was excluded from this analysis because she was not recorded within 100 m of oak or pine habitat. The two vegetation variables were correlated (Table 4.S1), so we summarized them using the first axis of a principal components analysis [PCA1, hereafter referred to as vegetation structure], which explained 75% of the variation. We also used the map of vegetation communities to determine whether oak or pine habitat dominated within a 100 m buffer around each focal female's location. Vegetation structure (PCA1) did not differ between habitat types (t-test,  $t_{72} = 0.4$ , P = 0.72,  $n = 53_{oak}$ ,  $21_{pine}$ ). Therefore, we included vegetation structure, habitat type, and their interaction in both models testing for an effect of vegetation and habitat on vocalization structure. We also included longitude as a covariate in the rattle rapidity model.

## Results

Bill morphology influenced the structure of rattle calls produced by Island Scrub-Jays. Females with longer bills produced faster rattles (bill length effect:  $F_{1,33} = 7.9$ , P = 0.009, Fig. 4.3a; bill depth effect:  $F_{1,33} = 2.0$ , P = 0.17, Fig. 4.3b; tarsus length effect:  $F_{1,33} = 0.4$ , P = 0.55), and females with deeper bills produced higher-frequency rattles (bill length effect:  $F_{1,20} = 0.9$ , P = 0.36, Fig. 4.3c; bill depth effect:  $F_{1,20} = 13.8$ , P = 0.002, Fig. 4.3d; tarsus length effect:  $F_{1,20} = 0.1$ , P = 0.83). Tarsus length, which was used as an index of body size, did not have an influence on vocal signal structure. Rattle calls also varied spatially across Santa Cruz Island. Consistent with the cultural evolution hypothesis, rattle rapidity varied along the east-west axis of the island ( $F_{1,74} = 5.3$ , P = 0.02, n = 75; Fig. 4.4). Center frequency, however, was not related to longitude ( $F_{1,45} = 0.6$ , P = 0.43, n = 46). The spatial pattern in rattle rapidity cannot be attributed to co-variation with vegetation structure or morphology (see correlation matrix in Table 4.S1). Longitude was moderately correlated with bill depth, but that morphological variable was not a significant predictor of rattle rapidity.

Our results did not conform with predictions of the acoustic adaptation hypothesis. Vegetation structure and habitat type were not significant predictors of variation in rattle rapidity (vegetation structure effect:  $F_{1,73} = 3.0$ , P = 0.09; habitat type effect:  $F_{1,73} = 2.1$ , P = 0.15; vegetation\*habitat interaction:  $F_{1,73} = 3.1$ , P = 0.08) or center frequency (vegetation structure effect:  $F_{1,44} = 0.1$ , P = 0.83; habitat type effect:  $F_{1,44} = 0.3$ , P = 0.60; vegetation\*habitat interaction:  $F_{1,44} = 1.4$ , P = 0.25). The effect of vegetation structure on rattle rapidity approached significance (P = 0.09), but the trend we observed (faster rattles in dense vegetation) was in the opposite direction predicted by the acoustic adaptation hypothesis (Boncoraglio & Saino 2007).

#### Discussion

Our findings are consistent with the hypothesis that the bill could act as a "magic" trait in Island Scrub-Jays because bill morphology is correlated with a vocal signal thought to be important for male-female interactions. Within the species' narrow geographic range (250 km<sup>2</sup>), *A. insularis* exhibits remarkable divergence in bill morphology across habitat types: individuals in pine habitat have longer, narrower bills than those in oak habitat (Chapter 3). Parallel morphological differences in the species' sister taxon, *A. californica*, are associated with

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tradeoffs in feeding efficiency on pine cones versus acorns (Peterson 1993; Bardwell *et al.* 2001) and are hence likely to be adaptive. Here we show that bill morphology is also correlated with the structure of a vocal signal associated with interactions between mated jays, the female rattle call (Fig. 4.3). Thus, we have data to support both of the criteria for a trait to qualify as a "magic" trait: namely, that it is (1) under divergent natural selection, and (2) directly related to factors that influence mate choice (Servedio *et al.* 2011). More research is needed to confirm that the bill of the Island Scrub-Jay is acting as a true "magic" trait, but the results presented here are an important first step.

One priority for future research will be to elucidate the mechanisms that underlie the relationship between bill morphology and vocal signal structure. Bill depth may influence the frequency of rattle calls because of constraints on maximum gape width (Westneat et al. 1993) or because bill depth influences the resonance properties of the nasal capsule (H. James, pers. comm.). However, hypothesized explanations are more difficult to develop for the relationship between bill length and rattle rapidity. Previous work has shown that larger-billed birds are constrained in terms of the pace with which they can produce trilled songs because they cannot move their bill as rapidly to modulate frequency changes (Podos 1997, 2001). The rattle calls produced by Island Scrub-Jays are similar to trilled songs in that they are also rapid, but they differ from those vocalizations in one important respect: frequency modulations do not occur within individual notes (see Fig. 4.2). Therefore, the same constraint does not apply. Further study will be needed to determine if the relationship between bill length and rattle rapidity is reflective of a morphological constraint. One alternate possibility derived from evolutionary models is genetic linkage (i.e., a "non-magic" trait complex; Servedio & Kopp 2012). The regulation of genes underlying variation in bill morphology is well-studied, and upregulation of

calmodulin has been shown to account for a substantial amount of variation in bill length (Abzhanov *et al.* 2006). Linkage between a gene that regulates calmodulin and a gene involved in the production of rattle calls could, therefore, account for why females with longer bills produce vocalizations that are more rapid. This is plausible alternative to morphological constraints given that vocal features like the rapidity of note production have been shown to have a genetic basis, even in species that exhibit plasticity due to learning (Marler & Sherman 1985; Slabbekoorn & Smith 2002a).

We also found that neutral processes were important in shaping variation in vocal signal structure, as rattle rapidity varied along the east-west axis of the island in accordance with spatial patterns of gene flow (Fig. 4.4; Chapter 3). This finding is consistent with a hypothesis of cultural evolution mediated by localized dispersal. Little is known about the timing of vocal learning in Island Scrub-Jays, but in a well-studied congener – the Florida Scrub-Jay (*A. coerulescens*) –evidence exists that a homologous female call, the "hiccup," does not change following natal dispersal (J. Fitzpatrick and R. Bowman, pers. comm.). Therefore, Island Scrub-Jays likely produce rattles that were learned in or around their natal location. Females have been observed dispersing up to 3.5 km from their natal territory (Chapter 3), so dispersal following learning likely contributes to some of the variation in rattle rapidity at any given location on the island.

Our findings were not consistent with multifarious selection acting across habitat gradients (i.e., parallel selection on morphological adaptations for foraging and acoustic adaptations for optimal sound transmission) because we found no evidence to support the acoustic adaptation hypothesis. Spatial autocorrelation in vegetation structure may be too fine-scale to allow for the evolution of vocal differences within the population (Richardson *et al.* 

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2014). Selection also may not be operating in accordance with variation in the acoustic environment. Rattle calls are only produced when females are in close proximity to their mate (typically within 30 m) and this distance may not be substantial enough to degrade the structure of rattle calls (Boncoraglio & Saino 2007). Alternatively, selection may not be acting to minimize signal degradation if male *A. insularis* use the structure of rattle calls to estimate distance to females during territorial disputes (McGregor & Krebs 1984; Fotheringham *et al.* 1997). The latter explanation seems less likely given that rattle calls are produced when males are nearby, but distance estimation could be advantageous in the more densely vegetated locations on the island (e.g., Fig. 4.S1) when visibility is limited.

Given that we were able to reject the acoustic adaptation hypothesis, we inferred that bill morphology had a direct effect on variation in vocal signal structure, either through mechanical constraints or genetic linkage. This link may be important for reducing gene flow between adaptively-divergent Island Scrub-Jays. We have evidence for non-random mating for bill length at the scale of the island (Chapter 3), a pattern that could be due to jays (1) actively selecting mates based on a visual cue like bill morphology (Ratcliffe & Grant 1983) or an acoustic cue like rattle calls (Ratcliffe & Grant 1985), or (2) simply pairing with nearby and hence morphologically similar individuals (Chapter 3). We also have evidence for a genetic discontinuity across the boundary between the largest pine stand and adjacent oak habitat (Chapter 3), which could also be due to assortative mating. Our results thus provide a tantalizing hint that vocal cues may contribute to non-random mating and a reduction in gene flow between habitats. Limited information, however, is available on the process of mate selection and territory acquisition in Island Scrub-Jays (Curry & Delaney 2002). Future research should address how

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individuals select mates and whether they can distinguish between rattle calls produced by birds with different bill morphologies.

An increasing body of evidence suggests that adaptive evolutionary divergence in the face of gene flow is more common in nature than is generally appreciated (Richardson *et al.* 2014). Divergent natural selection on "magic" traits or on loci in "non-magic" trait complexes could be an important mechanism for reducing gene flow between adaptively-divergent groups that are in close proximity (Servedio & Kopp 2012). The link between bill morphology and acoustic features is one mechanism that holds great promise for explaining microgeographic divergence in birds (Huber & Podos 2006; Badyaev *et al.* 2008), but similar mechanisms likely exist in other taxa. For instance, assortative mating could evolve as a by-product of divergent natural selection on wing coloration in Lepidoptera (Jiggins *et al.* 2001; Fordyce *et al.* 2002) and on body size and shape in freshwater fishes (Nagel & Schluter 1998; Langerhans *et al.* 2007) because those traits are used as cues during mate selection. Ultimately, more work is needed on a broad array of taxa to determine whether linkages between ecological traits and mating signals are an important factor driving microgeographic divergence within populations.

## Figures



**Figure 4.1** Recording locations for rattle calls produced by female Island Scrub-Jays (n = 54 in oak habitat, 21 in pine habitat) on Santa Cruz Island, California, USA. The baselayer is colored according to habitat type (light orange = oak, dark green = pine) and shows locations where vegetation height was greater than 0.5 m (habitats without oak or pine trees were excluded). The size of the dots corresponds to the number of individuals recorded in that location. Individuals recorded within 500 m of each other were combined for visualization purposes.



**Figure 4.2** Waveform (a) and spectrogram (b) for a "rattle" vocalization produced by a female Island Scrub-Jay.



**Figure 4.3** The influence of bill morphology on rattle rapidity (a, bill length; b, bill depth; n = 34) and center frequency (c, bill length; d, bill depth; n = 21) in Island Scrub-Jays. Females with longer bills produced faster rattles, and females with deeper bills produced higher-frequency rattles. The lines give the best linear fit to the data for significant relationships.



**Figure 4.4** Female Island Scrub-Jays on the western side of Santa Cruz Island had faster rattle calls than females on the eastern side of the island (n = 75). A longitude value of zero corresponds to the westernmost tip of the island. The line gives the best linear fit to the data.

# Supplementary tables and figures

**Table 4.S1** Matrix depicting the correlation between continuous covariates. The lower left triangular matrix shows the scatter plots (with 95% density ellipses) and the upper right triangular matrix shows the Pearson correlation coefficient (r) and the sample size (n) for each correlation. When examining individual cells, the variables in the plot/correlation can be found by looking in the vertical and horizontal directions. For example, longitude is the x-axis variable for all plots below the longitude label, and it is a variable in all of the correlation results listed to the right of the label. See the text for descriptions of each variable.

Longitude	r = 0.09 $n = 34$	r = -0.36 n = 34	r = 0.11 $n = 34$	r = -0.18 $n = 74$	r = -0.15 $n = 74$	r = -0.19 n = 74
	Bill length	r = 0.05 $n = 34$	r = -0.21 n = 34	r = 0.35 $n = 34$	r = 0.50 $n = 34$	r = 0.49 $n = 34$
		Bill depth	r = -0.09 n = 34	r = 0.04 $n = 34$	r = 0.00 $n = 34$	r = 0.02 $n = 34$
			Tarsus length	r = -0.10 n = 34	r = -0.28 n = 34	r = -0.22 n = 34
				Proportion vegetation	r = 0.50 $n = 74$	r = 0.87 $n = 74$
	· · · · · · · · · · · · · · · · · · ·	• • • • • •	•	in the second	Mean vegetation height	r = 0.87 $n = 74$
				i a i i santa	······································	Vegetation structure (PCA1)



**Figure 4.S1** Aerial images of locations where vocalizations were recorded (white dots) in oak chaparral habitat. These locations were chosen as examples to represent areas where vegetation is (a) dense and (b) sparse. Images were obtained from the Environmental Systems Research Institute (Redlands, California, USA).



**Figure 4.S2** Rattle rapidity (in notes per second, Hz) was not related to center frequency (in sound waves per second, Hz) in Island Scrub-Jays (n = 46).

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