ORIGINAL ARTICLE

Islands within an island: Repeated adaptive divergence in a single population

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Physical barriers to gene flow were once viewed as prerequisites for adaptive evolutionary divergence. However, a growing body of theoretical and empirical work suggests that divergence can proceed within a single population. Here we document genetic structure and spatially replicated patterns of phenotypic divergence within a bird species endemic to 250 km^2 Santa Cruz Island, California, USA. Island scrub-jays (*Aphelocoma insularis*) in three separate stands of pine habitat had longer, shallower bills than jays in oak habitat, a pattern that mirrors adaptive differences between allopatric populations of the species' mainland congener. Variation in both bill measurements was heritable, and island scrub-jays mated nonrandomly with respect to bill morphology. The population was not panmictic; instead, we found a continuous pattern of isolation by distance across the east–west axis of the island, as well as a subtle genetic discontinuity across the boundary between the largest pine stand and adjacent oak habitat. The ecological factors that appear to have facilitated adaptive differentiation at such a fine scale—environmental heterogeneity and localized dispersal—are ubiquitous in nature. These findings support recent arguments that microgeographic patterns of adaptive divergence may be more common than currently appreciated, even in mobile taxonomic groups like birds.

KEY WORDS: Adaptation, *Aphelocoma*, gene flow, morphological evolution, natural selection, population structure.

A long-standing debate in evolutionary biology has centered on the relative importance of geography and ecology as factors generating biological diversity (Mayr 1947; Maynard Smith 1966). Due to the homogenizing influence of gene flow (Lenormand 2002; Nosil and Crespi 2004; Postma and van Noordwijk 2005), geographic isolation of populations has traditionally been viewed as the primary scenario favoring evolutionary divergence (Mayr 1963; Via 2001; Bolnick and Fitzpatrick 2007), particularly in mobile taxonomic groups like birds (Coyne and Price 2000; Grant 2001). However, there is growing appreciation for the role of ecological variation, natural selection, and other processes in driving divergence, even at fine spatial scales within populations (Feder et al. 1988; Schluter 2000, 2009; Rundle and Nosil 2005; Shafer

and Wolf 2013). This has generated calls for more research to understand the conditions that lead to differentiation when gene flow and divergent selection are acting simultaneously (Bolnick and Fitzpatrick 2007; Richardson et al. 2014).

Evidence for adaptive divergence at microgeographic scales—that is, within the dispersal radius of the focal organism— is limited and has come from a mix of theory and empirical work (Richardson et al. 2014). Some models predict that multiple lineages can evolve within a single population when certain restrictive conditions are met (Maynard Smith 1966; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Bolnick 2006). These models were largely developed to test mechanisms that could give rise to separate species (sympatric speciation),

but they also apply to adaptive divergence within populations (Bolnick 2006). Unfortunately, there is uncertainty surrounding how commonly the model conditions are met in natural populations; sympatric speciation is challenging to demonstrate (Bolnick and Fitzpatrick 2007) and most studies of local adaptation have occurred at spatial scales that exceed the dispersal radius of the focal organism (Richardson et al. 2014). Nevertheless, a growing number of studies have documented adaptive evolutionary divergence within populations in the lab (reviewed by Rice and Hostert 1993) and in the wild (reviewed by Bolnick and Fitzpatrick 2007; Richardson et al. 2014), suggesting that fine-scale patterns of local adaptation—and even sympatric speciation—may be more common than generally assumed.

Theoretical and empirical work suggests that a variety of scenarios could allow for evolutionary divergence within a single population. Most organisms are exposed to heterogeneous environmental conditions at a variety of spatial scales, and adaptive differences may evolve at fine scales if (1) divergent selection is strong and/or (2) there are mechanisms acting to generate a pattern of nonrandom mating for the trait under selection (Rice and Hostert 1993; Bolnick and Fitzpatrick 2007; Richardson et al. 2014). The latter could occur if dispersal is nonrandom and individuals display a preference for environments that their phenotype is best suited for exploiting (matching habitat choice; Edelaar et al. 2008) or a preference for their natal habitat (Davis and Stamps 2004). It could also occur if individuals actively select mates that are phenotypically similar to themselves (assortative mating; Servedio et al. 2011). Yet another option involves spatially autocorrelated natural selection, combined with localized dispersal (isolation by distance); in this scenario, dispersal and mate selection are neutral processes, but individuals are more likely to disperse to an environment that is similar to their natal habitat (and mate with individuals there) simply because they disperse locally (Mallet et al. 2009). Despite the plausibility of these scenarios, relatively few empirical studies have tested for adaptive divergence at the spatial scale where gene flow is expected to operate across the landscape (Richardson et al. 2014; exceptions include, but are not limited to Blondel 1999; Milá et al. 2009; Kavanagh et al. 2010; Richardson and Urban 2013; Arnoux et al. 2014).

Here we test for microgeographic divergence in bill morphology in a New World jay (genus *Aphelocoma*). This clade of birds has served as a model system for the study of evolutionary diversification because 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 and Fitzpatrick 1984; Berg et al. 2011), and morphological traits (Pitelka 1951; Peterson 1993; McCormack and Smith 2008). Morphological changes have also occurred in association with transitions between habitat types. For instance, a survey of

western scrub-jays (*A. californica*) across western North America demonstrated that coastal populations located in oak (*Quercus* sp.) woodlands have shorter, deeper bills than inland populations located in forests composed of pinyon pine (*Pinus edulis*) and juniper (*Juniperus* sp.; Peterson 1993). These morphological differences are likely adaptive because western scrub-jays primarily feed on the seeds of masting trees during the fall and winter months (Curry et al. 2002) and there are trade-offs in feeding efficiency: long, shallow bills are more efficient for extracting seeds from pine cones but are less efficient for hammering and prying open acorns (Bardwell et al. 2001).

In the present study, we test for the same habitat-related pattern of bill divergence, but within the much smaller geographic range of the island scrub-jay (*A. insularis*), a species restricted to Santa Cruz Island (250 km^2) in southern California, USA. Island scrub-jays are most closely related to coastal populations of western scrub-jays, but the species has been evolving in isolation for approximately one million years (McCormack et al. 2011) with no evidence of ongoing gene flow with mainland *Aphelocoma* (Delaney and Wayne 2005). During that time, Santa Cruz Island has experienced marked changes in vegetation communities: coniferous forests predominated during the Pleistocene (Anderson et al. 2009), but oak (primarily island scrub oak, *Q. pacifica*) forms the majority of woody vegetation across the contemporary landscape (Junak 1995). Three relict stands of bishop pine (*P. muricata*) also form habitat "islands" of different sizes in the western (418 ha), central (224 ha), and eastern (21 ha) portions of the island (Walter and Taha 1999; Fischer et al. 2009; Fig. 1). As jay habitats, the spatial pattern of oak and pine represents a case of mosaic sympatry (Mallet et al. 2009)—rather than parapatry—because all of the pine habitat on Santa Cruz Island is within the potential dispersal radius of island scrub-jays in oak habitat (see Fig. S1). This situation presents an ideal opportunity to test the general question of whether a single population is capable of evolving adaptive differences in response to environmental heterogeneity because (1) repeated oak-pine transitions occur at small spatial scales, with no physical barriers to gene flow, and (2) the limited insular range precludes the effects of gene flow from elsewhere, an issue that can confound studies of adaptive divergence (Postma and van Noordwijk 2005).

We present morphological and genetic data that were collected from a quarter ($n = 565$) of the entire population of island scrub-jays ($N_c \sim 2300$ individuals; Sillett et al. 2012). The objectives of our study were twofold. First, we sought to determine whether island scrub-jays in pine habitat have longer, shallower bills compared to conspecifics in oak habitat, as has been found in western scrub-jays. Second, we looked for evidence of nonrandom mating in island scrub-jays, a critical requirement of sympatric divergence models. To do this, we tested for nonrandom mating with respect to variation in bill morphology, using morphological
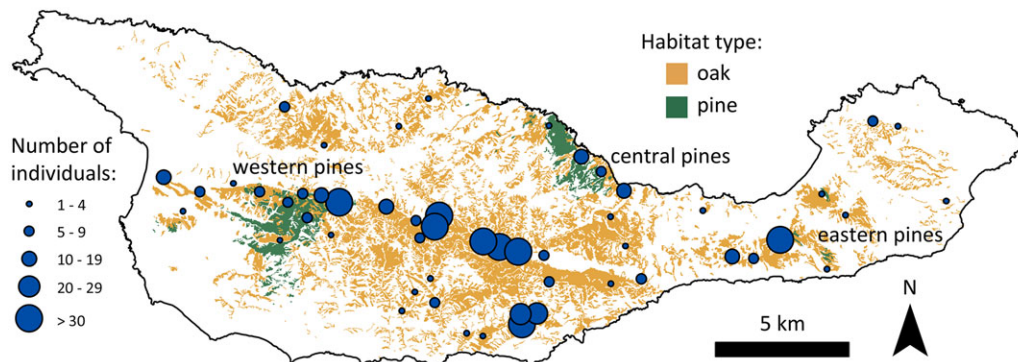
Figure 1. Locations where island scrub-jays were sampled across Santa Cruz Island (*n* = 565). The sizes of the dots correspond to the number of individuals sampled in that area; individuals within 1 km of each other were pooled for visualization purposes. The island is shaded according to habitat type. Note that the sample dots obscure the location of pine patches on the eastern side of the island due to their small size.

data from known breeding pairs. We also used genetic data from neutral molecular markers (microsatellites) to quantify the level of gene flow across the landscape, specifically testing whether the island scrub-jay population exhibits (1) panmixia, (2) isolation by environment (i.e., reduced gene flow between pine and oak habitats), and/or (3) isolation by distance (Sexton et al. 2014). In addition, we considered the potential for isolation by barrier (i.e., reduced gene flow between habitat fragments) because the habitat occupied by island scrub-jays is patchy (Sillett et al. 2012). This allowed us to differentiate between alternative scenarios that could lead to microgeographic divergence. For instance, isolation by environment would be consistent with scenarios that involve assortative mating or habitat-biased dispersal, whereas a strict pattern of isolation by distance would be consistent with a scenario involving spatially autocorrelated selection and localized dispersal.

Methods
STUDY SYSTEM
Island scrub-jays are resident passerine birds endemic to Santa Cruz Island, a small but ecologically diverse island that lies at the northern end of the Channel Island archipelago of southern California, USA. Despite being one of the most range-restricted birds in North America, the movement of individual island scrub-jays is far more localized than the bounds of the island. Territorial individuals have extremely small home ranges (3.5 ± 0.2 ha [mean $\pm$ SE], $n = 129$ [Caldwell et al. 2013]) that they occupy year-round; because of this, the vast majority of island scrub-jays live and breed in either oak or pine habitat (not both). Intense competition for space forces some individuals to exist as "floaters" until they can acquire a breeding territory (Collins and Corey 1994), but they still maintain home ranges that encompass approximately eight breeding territories (Mudry 2008). In addition, our limited

data on natal dispersal (see *behavioral data* below) indicates that individuals typically move short distances between their natal territory and the territory they eventually breed on (<1 km for males, $n = 18$; <4 km for females, $n = 4$; Fig. S1).

The species has a generalist diet similar to mainland *Aphelocoma* (Pitelka 1951; Curry et al. 2002) and consumes a variety of items ranging from plant matter to insects to small vertebrates (Curry and Delaney 2002). Acorns are a key component of their diet, especially during the nonbreeding season (Curry and 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 and 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 from oak trees that are intermixed with pine trees in that habitat type.

FIELD SAMPLING
We sampled 565 island scrub-jays from 2009 to 2011 at widely distributed locations across the island (Fig. 1). The maximum distance between sampled individuals was 29 km. 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 but two captured jays and stored in lysis buffer until DNA extraction. Genetic samples of 19 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 ±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 lengths were also measured with a ruler (to ±0.5 mm). To eliminate observer error, KML (author) 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 estimate repeatability (Lessells and 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 and 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 420 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. S2). The photos were used for morphometric analyses (see *morphological analyses* 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 (see Caldwell et al. 2013 for more details). KML measured both the male and the female (when they were adults) for 42 pairs to test for nonrandom pairing by bill morphology. We also documented 22 natal dispersal events, 18 of which were for males and four 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 *microsatellite genotyping* 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, using ArcGIS (ESRI 2011).

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), following manufacturer protocols. All samples were then genotyped at 12 variable microsatellite loci

(Table S1). Microsatellites were amplified in 10 μL multiplex reactions (Langin 2014), with two to five loci per reaction, using QIAGEN's type-it microsatellite PCR kit and a Vapo-Protect Mastercycler 6321 (Eppendorf, Hauppange, NY). Loci in the same reaction were distinguished from one another by unique fluorescent labels or by predetermined 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). 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. This was necessary because—while males are generally larger than females—the only way 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 and Ellegren (1999). The PCR products were run on a 2% agarose gel with Tris-borate-EDTA (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% (two of 210 known-sex birds were sexed incorrectly, both of which were female), which is low compared to other avian studies that used molecular sexing techniques (Robertson and Gemmell 2006).

MORPHOLOGICAL ANALYSES
We used spatially explicit linear models (Ward and 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 (PCAs) on tarsus, wing, and tail lengths, 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. 1). We used ArcGIS software 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 ε and ξ represent the nonspatially autocorrelated and spatially autocorrelated portions of the error term, respectively, λ 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 nonindependence between individuals captured within close proximity, and was performed using the *spdep* package in R (R Core Team 2012). Three parameters (β 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 and 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.

When a morphological variable differed between habitats, we ran additional models to test for the presence of spatial patterns within oak habitat. 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). We applied a linear transformation to rescale the distance-to-pine variable 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 and 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. 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 semilandmarks (Bookstein 1991) and align the specimens, and used MorphoJ software (Klingenberg 2011) to perform a Procrustes fit and generate covariance matrices. This method removed nonshape-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 10,000 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. Genetic parentage was confirmed using CERVUS software (at a 95% confidence level) because island scrub-jays engage in extra-pair copulations (Delaney 2003; Desrosiers 2014). Individuals were included in the analysis when we had measurement data for the offspring and at least one parent ($n = 26$ offspring from 12 families). We estimated the proportion of phenotypic variance explained by the pedigree—that is, 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 burn-in and a thinning of 500. Sex and age were included as fixed effects in the models. The results we report 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 0.43 to 0.82 (0.59 ± 0.05 [mean $\pm$ SE], $n = 9$) for bill length and 0.10–0.71 (0.42 ± 0.08, $n = 9$) 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 (i.e., h^2 is nonzero).

GENETIC ANALYSES

We tested for the presence of null alleles and large-allele dropouts using MICRO-CHECKER software (Van Oosterhout et al. 2004). We then used GENEPOP software (Raymond and Rousset 1995) to test for linkage disequilibrium and to calculate summary statistics for each locus.

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, 10,000 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 and 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* (in contrast, F_{ST} would be used to compare *populations*).

The second method we employed to test for spatial genetic structure was a spatial PCAs (sPCAs) (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 sPCAs and to conduct permutation tests (with 10,000 iterations) on the resulting axes to test for the presence of both global and local genetic structure.

Both the Mantel test and the sPCAs 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 and 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 (Jombart et al. 2008).

The 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 environmental 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. In contrast, we modeled habitat fragmentation 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 nine 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).

Results
BILL MORPHOLOGY

We found that the size and shape of island scrub-jay bills differed between pine and oak habitats on Santa Cruz Island. Birds captured in oak habitat ($n = 351$) had shorter, deeper bills compared to birds captured in pine habitat ($n = 112$; habitat-related cumulative AIC_c weights for bill length and bill depth were both >0.95; Table S2), a pattern that was apparent in all sex and age groups. Both of these traits were heritable in island scrub-jays (h^2 estimates [and 95% credible intervals] for bill length: 0.57 [0.26–0.85]; and bill

Table 1. 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 (see Table S2).

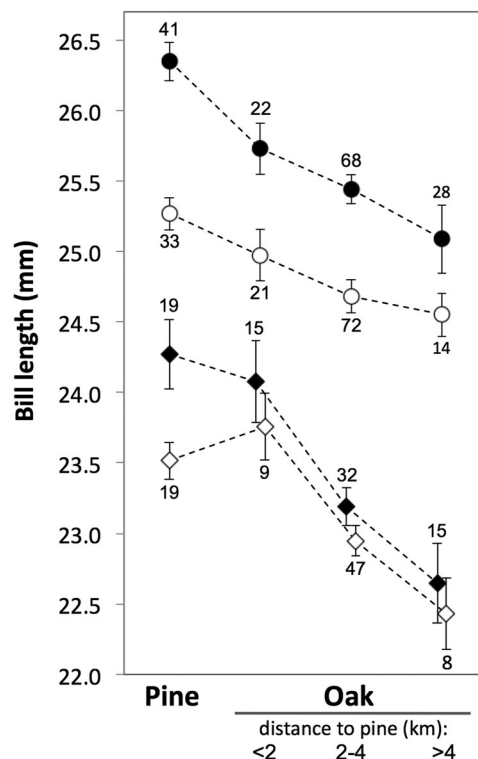| Trait	Parameter	Model-averaged β-value
Bill length	Sex	1.95 (1.86–2.03)
	Age	0.67 (0.59–0.76)
	Western pines	0.64 (0.49–0.79)
	Central pines	0.85 (0.62–1.07)
	Eastern pines	0.83 (0.63–1.03)
Bill depth	Sex	0.52 (0.50–0.55)
	Age	0.16 (0.14–0.18)
	Western pines	−0.07 (−0.11 to −0.04)
	Central pines	−0.16 (−0.22 to −0.10)
	Eastern pines	−0.10 (−0.16 to −0.05)
Body size	Sex	2.34 (2.27–2.41)
	Age	1.40 (1.33–1.47)
	Western pines	−0.04 (−0.11 to 0.02)
	Central pines	−0.01 (−0.11 to 0.08)
	Eastern pines	0.21 (0.13–0.29)

Positive β-values for sex, age, and pines indicate that males, adults, and pine birds, respectively, had larger morphological measurements.


Figure 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 ± 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).

depth: 0.40 [0.10–0.76]). Furthermore, the habitat-related differences in bill measurements were remarkably consistent across all three of the island's primary pine stands (Table 1). These results cannot be attributed to habitat-related differences in overall body size because island scrub-jays were not consistently larger (or smaller) in pine habitat (Table 1).

Geometric morphometric analyses also revealed habitat-related differences in bill shape while controlling for variation in overall bill size. The shape of island scrub-jay bills differed significantly between oak and pine habitat for adult males (permutation test, *Mahalanobis distance* (*M*) = 2.35, *P* < 0.0001), young males (*M* = 2.60, *P* < 0.0001), adult females (*M* = 3.15, *P* < 0.0001), and young females (*M* = 2.33, *P* < 0.0001; Fig. S3). These shape differences reflected variation in the length to depth ratio—with oak individuals exhibiting shorter, deeper bills—and not habitat-related variation in curvature at the tip of the bill (see Fig. S4).

Bill differences were not only apparent across the pine-oak habitat boundary, but they were also apparent within the matrix of oak habitat. After excluding individuals captured in pine habitat, we found that island scrub-jays had significantly shorter bills when they were captured further away from pine habitat (log-likelihood ratio test, *T* = 18.6, *P* < 0.001; Fig. 2). Bill depth, however, did not vary among oak birds in relation to distance-to-pine (*T* = 0.23, *P* > 0.05).

Island scrub-jays mated nonrandomly with respect to bill morphology. Longer billed females were more likely to mate

with longer billed males ($r^2 = 0.32$, *P* < 0.0001, *n* = 42; Fig. 3), even when the analysis was restricted to oak habitat ($r^2 = 0.22$, *P* = 0.006, *n* = 33). We found no evidence for nonrandom mating with respect to bill depth ($r^2 = 0.01$, *P* = 0.48, *n* = 42).

SPATIAL GENETIC STRUCTURE

Both analyses used to test for the presence of spatial genetic structure rejected the null hypothesis of panmixia: genetic distance between individuals (Rousset 2000) increased with geographic distance (Mantel test, *r* = 0.11, *P* < 0.0001; Fig. S5), and our sPCAs detected significant global genetic structure (permutation test, *P* = 0.01) but not local genetic structure (*P* = 0.18). An additional landscape genetics analysis—which tested factors hypothesized to influence variation in the first sPCA axis—indicated that the primary pattern of spatial genetic structure in island scrub-jays was due to variation across longitudes (*cumulative AIC_C weight* = 1.00) and habitat types (0.95), with no effect of latitude (0.01) or
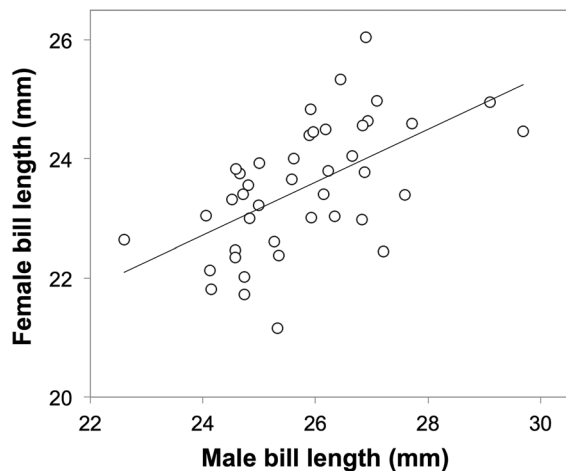
Figure 3. Island scrub-jays mated nonrandomly with respect to bill length. Each point represents a pair of breeding birds (*n* = 42). All individuals were measured as adults. The same result was obtained when controlling for body size.

habitat fragmentation (0.02; Table S3). The effect of habitat type appeared to be largely restricted to the western pine stand because the 95% confidence intervals for the top model's β estimates included zero for the central and eastern pine stands, but did not include zero for the western pine stand (Table S4). Spatial variation in the genetic structure of island scrub-jays, therefore, was primarily driven by a strong east–west gradient across the 35 km length of Santa Cruz Island, as well as a genetic discontinuity associated with the western pine stand that had an effect size equivalent to genetic variation across 3 km of longitude (see Fig. 4).

Discussion

The capacity for a single population to diversify and adapt to multiple ecological opportunities is a fundamental assumption of theory explaining sympatric speciation (Via 2001; Bolnick and Fitzpatrick 2007; Mallet et al. 2009). Yet, adaptive divergence is thought to be rare within populations due to constraints imposed by gene flow and recombination (Mayr 1963; Lenormand 2002; Kawecki and Ebert 2004). Here we document a striking pattern of microgeographic divergence within one of the most narrowly distributed bird species in North America. Island scrub-jays in all three of the island's pine stands had longer, shallower bills than jays in oak habitat, a pattern consistent with predictions based on a hypothesis of trophic adaptation as documented in western scrub-jays (Peterson 1993; Bardwell et al. 2001). Remarkably, the difference in bill length between pine jays and jays in oak more than
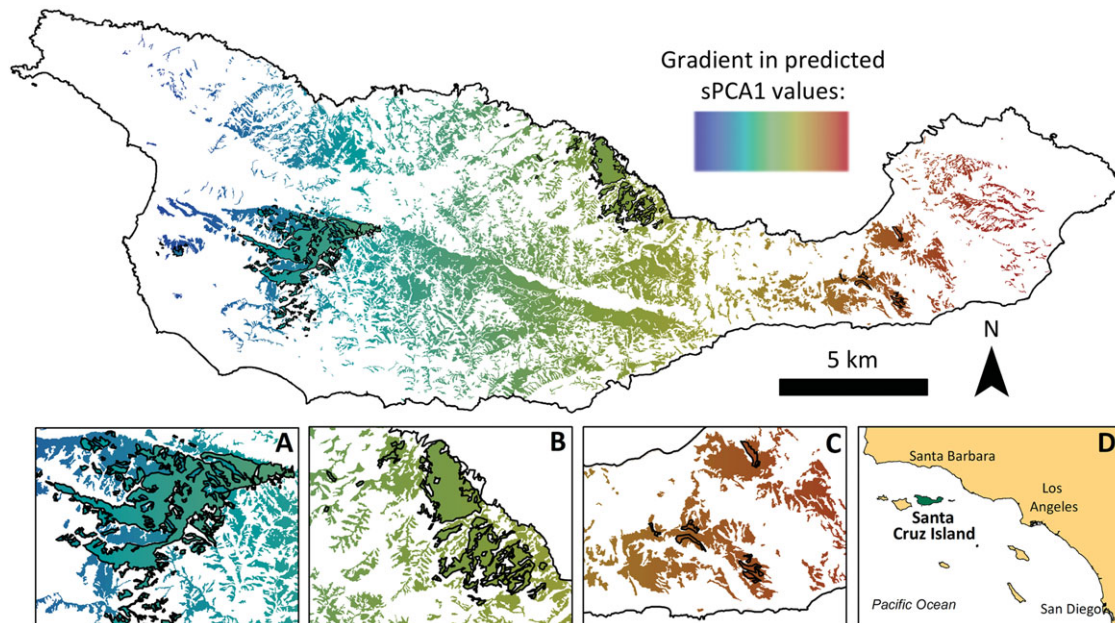
Figure 4. The island scrub-jay population exhibited spatial genetic structuring across Santa Cruz Island. Regions outlined in black represent pine habitat and the remaining colored regions represent oak habitat. The color gradient is a continuous representation of the predicted genetic surface for the 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; jays are expected to have a similar genetic composition (at neutral molecular markers) if they are located in areas with similar colors. The magnitude of genetic differentiation is such that the probability of allelic identity between an individual on the east versus the west side of the island is 10% less than between two neighboring individuals (Fig. S5). 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 (in green) relative to the other Channel Islands and mainland California.

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 morphological differences are unlikely to be a product of drift because the same pattern—long, shallow bills in pine habitat—occurs repeatedly not only within the island but also among mainland populations of *Aphelocoma* jays. Although selection was not quantified directly, divergent natural selection on bill morphology is the most likely explanation for the repeated patterns (see further discussion below). These findings provide additional support for recent arguments that microgeographic patterns of local adaptation are more common than currently appreciated (Richardson et al. 2014), even in mobile taxonomic groups like birds.

Our morphological and genetic data indicate that island scrub-jays in pine and oak habitat do not represent divergent lineages. Instead, the morphological differences appear to have originated in situ and been maintained in the face of some gene flow between habitat types. Bill morphology did not display a sharp transition across the pine-oak boundary, as would be the case for a trophic polymorphism (Skulason and Smith 1995). Rather, bill length declined gradually with distance from pine habitat, a clinal pattern that is consistent with a scenario of adaptive divergence with gene flow (May et al. 1975; Moore and Hendry 2005). Furthermore, island scrub-jays in all three of the island's pine stands were genetically more closely related to jays in neighboring oak habitat than they were to jays in the other pine stands. We did find evidence for a subtle genetic discontinuity across the boundary of the western—and largest—pine stand, which suggests a further reduction in gene flow between these habitats. Such a reduction could be due to assortative mating based on bill morphology (Servedio et al. 2011), natural selection against dispersing individuals (Nosil et al. 2005), and/or habitat selection based on natal origin (Davis and Stamps 2004). Unfortunately, few data are available on the demography and localized movements of island scrub-jays in the vicinity of pine habitat. Future work will be needed to understand the mechanism underlying the apparent presence of an environmental barrier to gene flow at the boundary of the western pine stand.

The observed habitat-related differences in bill morphology are consistent with predictions based on evolved trophic adaptations in western scrub-jays, but environmentally induced phenotypic plasticity could contribute to these patterns. Bill morphology has a well-documented genetic basis in other bird species (Smith and Zach 1979; Boag 1983; Abzhanov et al. 2006; Knief et al. 2012) and is heritable in island scrub-jays as well (see results). 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 modes of plasticity are unlikely to explain habitat-related differences in bill morphology for the following reasons. First, bill length in island scrub-jays does vary seasonally, but seasonal variation is consistent across habitats (Fig. S6). We therefore found no evidence that bill wear is habitat dependent (e.g., due to prying open pine cones). Second, neither mode of plasticity can account for the strong relationship between bill length and distance-to-pine within oak birds (Fig. 2). If phenotypic plasticity due to variation in growth or wear was the primary cause of habitat-related differences, we would expect it to generate an abrupt change in bill morphology across the pine-oak habitat boundary and no spatial pattern within habitat types (see Fig. S7A). Similarly, if differences were due to developmental plasticity, we would expect a pattern with intermediate phenotypes near the oak-pine boundary—because some individuals may have dispersed between habitat types—and no spatial pattern beyond the zone of potential dispersal between pine and oak habitats (see Fig. S7B). In contrast to these expectations, 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. 2). Hence, although there is a plastic component to variation in bill morphology, neither of the two modes of plasticity can account for the pattern of spatial variation in bill length within oak birds.

Another potential explanation for the pattern we observed involves matching habitat choice (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, especially for males. We documented natal dispersal distances for 18 male island scrub-jays that hatched in oak habitat; all of them dispersed less than a kilometer from their natal territory (Fig. S1). 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). Therefore, these data suggest that, at least for males, most island scrub-jays that hatch in oak habitat do not have the option of selecting pine habitat. Furthermore, the matching habitat choice hypothesis predicts that the greatest habitat-related difference in bill morphology for males should occur within 1 km of the transition between pine and oak habitat (i.e., within the spatial scale at which habitat-based sorting could occur; see Fig. S7C), but we found the reverse: island scrub-jays had shorter bills the further they were from pine habitat (Fig. 2).

Thus, matching habitat choice is unlikely to explain the patterns documented here.

Consistent with models of sympatric divergence (Bolnick and Fitzpatrick 2007), we found that island scrub-jays mated nonrandomly 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 (Fig. 3). This result may in part be due to active mate selection based on bill morphology (Langin 2014). However, we hypothesize that limited dispersal across the landscape (i.e., isolation by distance; Fig. 4) and spatial variation in bill morphology (Fig. 2) combine to explain much of the pattern of nonrandom mating. In other words, long-billed females have a higher likelihood of mating with long-billed males because that is the most common bill morphology in their local neighborhood. This explanation is congruent with the recent perspective that localized dispersal (i.e., isolation by distance) may be an important mechanism underlying patterns of nonrandom mating within populations that are exposed to spatially divergent selection (Mallet et al. 2009).

Environmental heterogeneity and localized dispersal appear to be the most important ecological factors driving adaptive divergence in this system; however, the unique ecology of islands may have played a supporting role. Fewer bird species occur on Santa Cruz Island compared to similar habitats on the California mainland (Yeaton 1974), so island scrub-jays may have access to a wider ecological niche than their mainland congener. Strong intraspecific competition may have also favored fine-scale evolutionary diversification on the island, as island scrub-jays occur at higher densities than western scrub-jays (Yeaton 1974) and their habitat appears to be saturated with territory holders (Collins and Corey 1994). Yet another possibility is that gene flow is more localized on the island because, as has been found for other insular species, selection pressures may have favored the evolution of reduced dispersal abilities (Blondel 2000). Indeed, lower dispersal is cited as the reason why blue tits (*Cyanistes caeruleus*) on the Mediterranean island of Corsica are locally adapted to two divergent habitat types at a microgeographic scale (Blondel 1999; Charmantier et al. 2004; Porlier et al. 2012), whereas mainland populations are not (Blondel et al. 2006). Data on natal dispersal are too sparse to enable a formal comparison of dispersal distance across *Aphelocoma* jays. Nevertheless, a preliminary analysis comparing spatial genetic structure in island scrub-jays (data presented here) and western scrub-jays (J. McCormack, unpubl. data) is consistent with this hypothesis: isolation by distance was detectable at a finer spatial scale on Santa Cruz Island than on the California mainland (K. Langin et al., unpubl. analysis). Thus, factors such as competitive release, strong intraspecific competition, and selection for lower dispersal may increase the likelihood that insular populations will evolve fine-scale patterns of adaptive divergence.

Beyond the evolutionary implications of this work, the findings reported here also have application in the conservation and management of island scrub-jays. The species was recently uplisted to "vulnerable" on the IUCN Red List (IUCN 2012) because of its narrow geographic range, small population size, and concerns about the potential for short-term threats such as fire and the arrival of West Nile virus (Boyce et al. 2011; Morrison et al. 2011; Sillett et al. 2012). From a long-term perspective, the species is also at risk due to climate change because island scrub-jays will not have the option of tracking latitudinal changes in vegetation communities and instead will be required to adapt to changes in situ. Therefore, the retention of maximal levels of genetic diversity may be especially important for future population persistence (Crandall et al. 2000; Hoffmann and Sgrò 2011). The island scrub-jay has lower levels of neutral genetic diversity than western scrub-jays on the California mainland (Delaney and Wayne 2005; Langin 2014) and the genetic diversity that it does have is apportioned spatially across the island (Fig. 4). In light of these results, conservation plans should not treat island scrub-jays as one homogenous group. Instead, they should consider managing the species at a microgeographic scale, so as to conserve intraspecific variation that is associated with different regions and habitat types within the island.

Conclusions

Our results demonstrate repeated patterns of microgeographic divergence within one of the most narrowly distributed bird species in North America. 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 likely reflect the maintenance of locally adapted ecotypes, rather than a case of nascent speciation (Bolnick 2006). Nevertheless, our results provide empirical support for the conditions used in models that invoke divergent selection and nonrandom mating as drivers of within-population divergence and sympatric speciation (Maynard Smith 1966; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Bolnick 2006).

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 (Richardson et al. 2014). 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 such as the island scrub-jay, which often have low genetic diversity and cannot disperse to track environmental changes.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. All of the pine habitat on Santa Cruz Island is within the potential dispersal radius of island scrub-jays in oak habitat.

Figure S2. Standardized bill photograph, showing an island scrub-jay, with a size standard affixed to its lower mandible, biting on a dowel.

Figure S3. 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).

Figure S4. Bill shapes represented by the canonical variate (CV) scores in the geometric morphometric analysis.

Figure S5. A comparison of pairwise genetic distance (Rousset's *a* [Rousset 2000]) across six geographic distance categories: 0–5 km ($n = 72,801$), 5–10 km ($n = 51,168$), 10–15 km ($n = 24,183$), 15–20 km ($n = 7792$), 20–25 km ($n = 2018$), and 25–30 km ($n = 241$).

Figure S6. 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 $\times$ habitat interaction, $P = 0.88$).

Figure S7. 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).

Table S1. Summary statistics for the microsatellite loci.

Table S2. 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 lengths).

Table 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).

Table S4. β-values (and 95% confidence intervals) estimated for parameters in the top model of the landscape genetics analysis (Table S3).