

Genetic and ecological differentiation in the endemic avifauna of Tiburón Island

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Tiburón Island is a land-bridge island in the Gulf of California, separated from mainland Sonora by 3 km. The shallow channel (13 m) separating the island and mainland is thought to have formed 10 000 years ago. Although the majority of avian resident species are not taxonomically differentiated, six species are represented by endemic subspecies (cactus wren, gila woodpecker, black-tailed gnatcatcher, Gambel's quail, canyon towhee, northern cardinal), of which all but one (black-tailed gnatcatcher) possess a pallid, ash-gray coloration compared to those on the mainland. We compared mtDNA sequences of five of the endemic subspecies (we lacked samples of northern cardinal) and one more widespread subspecies (verdin) present on the island from sequences previously published for mainland populations. For most populations, we discovered no genetic differentiation between the island and the mainland, thus questioning the taxonomic validity of the endemic subspecies. The canyon towhee and the verdin showed significant mitochondrial DNA differentiation, although neither was reciprocally monophyletic. We modeled the ecological niche for the mainland populations of the study species (plus the curve-billed thrasher, which was studied earlier) and determined if species' occurrence on the island was predicted. We found no ecological differences for the four species that showed no genetic differences and one of the species that did (verdin). In contrast, some ecological differentiation was detected for the canyon towhee and the curve-billed thrasher. We conclude that the ecological differences leading to paler plumages in Tiburón Island endemics are sufficiently subtle as to not be discovered in our ecological models, although they are likely influenced by variation in rainfall, temperature and the vegetation. In addition, the black-tailed gnatcatcher is not paler, and therefore might respond to different ecological variables. We simulated sequence data and showed that if the populations on Tiburón Island have been isolated for 10 000 years, there ought to be greater differences than we observed for black-tailed gnatcatcher, Gambel's quail, cactus wren, and gila woodpecker, suggesting that there has been gene flow connecting the mainland and island populations. If so, then the paler coloration of these Tiburón Island subspecies (excluding black-tailed gnatcatcher) has been maintained by natural selection despite gene flow. In any case, the pale coloration apparently evolved within the past 10 000 years.

The study of island biotas has highlighted many aspects of the evolutionary process (Darwin 1859, Wallace 1860). The discrete isolation of islands 'controls' for such factors as population size, distance from mainland as well as maximum time since isolation. Nonetheless, many questions related to the evolution of island birds remain (Ricklefs and Bermingham 2007). For example, why do populations on islands often differ in plumage traits more often than in size (Grant 2001), which is more typical of continental species? It seems clear that the more isolated an island is, the greater the distinctiveness of its endemic inhabitants (Grant 2001). Most studies have been performed on remote island groups such as the Hawaiian, Philippine and Galapagos archipelagoes. Consequently, study of evolutionary patterns on offshore islands has been overlooked (Cook et al. 2001). Study of the patterns of genetic and ecological differentiation in birds

inhabiting offshore islands reveals evolutionary processes at a more local scale, and potentially offers a more direct comparison with continental faunas.

Tiburón Island is the largest island (ca 1208 km²) in Mexico and lies in the Gulf of California (Fig. 1) between 28°45'–29°15'N and 112°12'–112°36'W; it is separated by only three km from its nearest point to the coast of Sonora. The island has a complex geological origin by faulting, uplift, and erosion during the Miocene–Pliocene (12–5 Ma). The ocean channel that separates the island from the mainland ('Canal del Infiernillo') reaches a maximum depth of 13 m, and climatic fluctuations potentially resulted in intermittent connections and temporary land bridges since its formation (Ferrusquía-Villafranca and González-Guzmán 2005). It is thought that Tiburón Island was last isolated 10 000 years ago (Gastil et al. 1999, Cody and Velarde 2002, Lowlor et al. 2002).

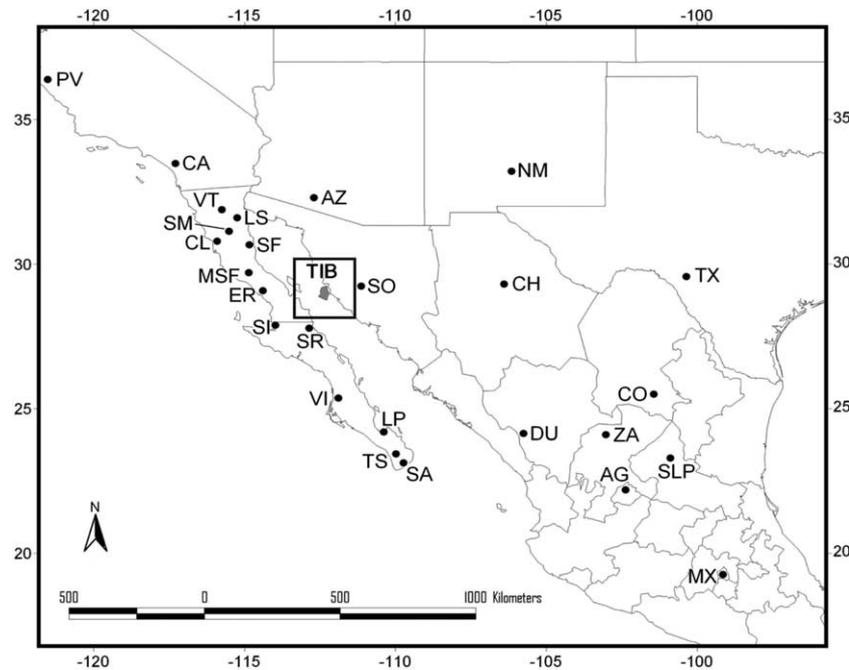


Figure 1. Geographic position of Tiburón Island (TIB) and surveyed mainland localities: PV (Palo Verde), CA (Salton Sea), LS (Laguna Salada), VT (Valle Trinidad), SM (Ejido San Matías), CL (Camalu), SF (San Felipe), MSF (Misión San Fernando), ER (El Rosario), SI (San Ignacio), SR (Santa Rosalía), VI (Villa Insurgentes), LP (La Paz), TS (Todos Santos), SA (Santa Anita), AZ (near Tucson), NM (southern New Mexico), TX (Near San Antonio), SO (Sonora, near Tecoripa), CH (east of city of Chihuahua), DU (north of Durango City), ZA (near city of Zacatecas), CO (Coahuila north of Saltillo), SLP (San Luis Potosí near Río Verde), AG (north of Aguascalientes City), MX (near Mexico City).

Thus, it is considered today to be a young, shallow-water land bridge island with Pleistocene connections to the mainland (Cody et al. 2002).

The major vegetation type of the Gulf of California coast region including Tiburón Island is thorn scrub (Cody et al. 2002). However, other diverse but relatively local vegetation types contribute to a diversity of plants and animals among the islands in the Gulf of California. Tiburón Island contains 298 species of plants that are also present on the mainland, but 96 that are not present on any other Gulf island (Bourillón et al. 1988, Cody et al. 2002). One plant subspecies (Cody et al. 2002), one beetle species (Sánchez-Piñero and Aalbu 2002) and 10 subspecies of mammals (López-Forment et al. 1996, Lowlor et al. 2002) have been described from the island. The lack of endemic reptiles on Tiburón Island contrasts with other islands in the Gulf region, in which several have high levels of endemism in mammals and reptiles (Cody and Velarde 2002, Murphy and Aguirre-León 2002). Thus, Tiburón Island provides a useful context in which to study differentiation in offshore islands.

Given its close proximity to the mainland, one might expect limited or no differentiation of birds on Tiburón Island. Indeed, most of the 137 bird species found on Tiburón Island (Townsend 1923, van Rossem 1932, 1945, Vaurie 1953, Rojas-Soto et al. 2002) are not taxonomically distinct, and in general the avifauna of the island is a subset of the adjacent Sonoran Desert avifauna (van Rossem 1932, Banks 1969, Jehl and Everett 1985, Cody and Velarde 2002). More unexpectedly, the island harbors seven endemic subspecies (Friedmann et al. 1950, Miller et al. 1957, Rojas-Soto et al. 2002, Dickinson

2003): *Callipepla gambelii pembertonii* Gambel's quail, *Melanerpes uropygialis tiburonensis* gila woodpecker, *Campylorhynchus brunneicapillus seri* cactus wren, *Poliophtila melanura curtata* black-tailed gnatcatcher, *Toxostoma curvirostre insularum* curve-billed thrasher, *Cardinalis cardinalis townsendi* northern cardinal and *Pipilo fuscus jamesi* canyon towhee. Descriptions of these taxa were mainly based on differences in plumage color (Townsend 1923, van Rossem 1930a, 1930b, 1932, 1942). With regard to the endemic subspecies, van Rossem (1932, p. 127) stated that "all have developed, to a degree unequalled in any other races of these species regardless of their environment, a pallid, ash-gray coloration." Although the description of an avian subspecies as pallid in coloration is not novel, the concordance of subspecies possessing this characteristic across such a small distance suggests a common response to an environmental gradient. The one exception is the black-tailed gnatcatcher, which is described as being slightly smaller with a browner, darker coloration (van Rossem 1932). Most of these endemic forms have mainland conspecific populations directly across the Canal del Infiernillo, although the nearest canyon towhee population to Tiburón Island is ca 200 km to the south.

We gathered mitochondrial DNA (mtDNA) sequences for five of the seven endemic subspecies (Table 1) and for a more widespread subspecies (verdin, *Auriparus flaviceps fraterculus*) present on this island. We compared mtDNA sequences from specimens collected on Tiburón Island to those previously published for mainland populations (Zink and Blackwell-Rago 2000, Zink et al. 2001) to determine if the subspecific (i.e. phenotypic) endemism reflects the

Table 1. Summary of individuals sequenced (n) from Tiburón Island and the mainland, number of haplotypes on Tiburón Island, phenotype of Tiburón subspecies and description of general pattern of genetic and ecological variation.

Species	n Tiburón Island/ mainland	No. of haplotypes	Phenotype of Tiburón subspecies	Differentiated from mainland? Genetically Ecologically	
<i>Polioptila melanura</i>	5/34	2	browner	no	no
<i>Campylorhynchus brunneicapillus</i>	5/60	1	pallid, ash-gray	no	no
<i>Pipilo fuscus</i>	5/28	2	pallid, ash-gray	yes	partially
<i>Callipepla gambelii</i>	5/3	2	pallid, ash-gray	no	no
<i>Auriparus flaviceps</i>	5/45	2	grayer	yes	no
<i>Melanerpes uropygialis</i>	5/15	2	paler	no	no
<i>Toxostoma curvirostre</i>	4/22	4	pallid, ash-gray	no	partially

genetic pattern of differentiation. We also compared geographic distributions to a predictive distributional model of ecological niches to explore intraspecific niche evolution and its potential relationship to the general pattern of paleness observed in Tiburón Island endemics.

Methods

Sampling and DNA analyses

Because Tiburón Island is a Biosphere Reserve our field time was restricted and we were authorized to collect a limited number of specimens. Tissues were stored in room temperature buffer (Seutin et al. 1991) and voucher specimens (study skins) from Tiburón Island were deposited at the Museo de Zoología, Facultad de Ciencias, Univ. Nacional Autónoma de México (MZFC). We used mtDNA because of the presumed recent isolation of populations and its relatively rapid coalescence time (Zink and Barrowclough 2008, Barrowclough and Zink 2009). Previously published sequences were downloaded from GenBank (U75574, AF287260-228, AF287493-629) for black-tailed gnatcatcher, cactus wren, canyon towhee and verdin. For Gambel's quail and gila woodpecker, we sequenced specimens obtained in Sonora and in New Mexico. These samples represent several individuals collected from widely spaced localities across the range of the complex (Zink and Blackwell-Rago 2000, Zink et al. 2001).

We extracted template DNA using Qiagen Dneasy Blood and Tissue Kit, according to manufacturer's protocol. We performed PCR using the following primers: for NADH dehydrogenase subunit 2 (ND2)–L–MET (J. Groth pers. comm.) and ASN (Pereira and Baker 2004), yielding the complete gene (1041 bp); for the control region I (CRI)–ND6E (Edwards 1993) and HCR4 (Tarr 1995); for control region II (CRII)–LCR4 and H1248 (Tarr 1995) and PasserCRF (3'–TACCTAGGAGGTGGGCGAAT–5') and PasserCRR (3'–CCCAAACATTATCTCCAAAA–5') (B. McKay pers. comm.); for cytochrome-b (CYTB)–L14841 (Kocher et al. 1989) and H4a (Harshman 1996). The described markers and primers were used for all groups except for the California quail, in which we sequenced just 304 bp of ND2 and 1148 bp of Cyt b.

For PCR, we used MJ Research PTC-100 thermocyclers with the following protocols: ND2 and Cyt b–15 min at 95°F, followed by 35 cycles of 30 s at 95°F, 30 s at 52°F, and 1 min at 72°F, concluding with a 10 min extension at 72°F and a hold of 4°F; CRI and CRII–15 min at 95°F,

followed by 35 cycles of 1 min at 95°F, 1 min at 50°F, and 1 min at 72°F, concluding with a 10 min extension at 72°F and a hold of 4°F; for primers PasserCRF and PasserCRR, the cycles were modified to 30 s at 95°F, 30 s at 55°F, and 1 min at 72°F. We used 25 µl reaction volumes, which included 0.125 Qiagen HotStarTaq, 1 µl of 10 mM solution of each primer, 2 µl of 25 mM magnesium chloride, 1 µl of a solution containing 10 mM solution of each dNTP, 2.5 µl of 10 × reaction buffer, and 2–3 µl of template. We purified PCR products using Qiagen Qiaquick PCR Purification Kit, following the manufacturers protocol. Purified products were sequenced on ABI 3130xl or 3730xl automated sequencers using Big Dye Terminator Chemistry 3.1 and appropriate primers (listed above) for each region. Chromatograms were aligned with the aid of Sequencher 4.6. Genbank accession no. are DQ241251 to DQ241298.

To explore the relationships of haplotypes from Tiburón Island, we used the TCS software (Clement et al. 2000) to construct parsimony networks. Because of small sample sizes, we did not compute population genetic measures.

Simulations

To predict the genetic characteristics of a population after 10 000 years of isolation, we simulated DNA sequence evolution using the software Simcoal (Excoffier et al. 2000). First, it was necessary to estimate the size of island and mainland populations. Based on our field work on Tiburón (ORRS pers. obs.), we concluded that the maximum population census size of any of our study species was ≤800 individuals. This is consistent with van Rossem's (1932, p. 122) view that "As in most desert areas we found birds to be uncommon not only as to species but as individuals." Given that the effective size of a population is much less than the census size, we arbitrarily chose a value of 500 for Tiburón, and assumed that the population on the mainland was 10 times larger, or 5000. Palstra and Ruzzante (2008) found that the ratio of N_e/N was ca 0.14 for many species, whereas our ratio was 0.63. We chose the higher ratio because we did not wish to underestimate N_e . We then simulated 1000 data sets of 400 bp assuming that the populations were isolated 10 000 years ago without subsequent gene flow, sequence evolution occurred at 2% per million years (Ma), and that we sampled 5 individuals from Tiburón and 20 from the mainland (after the 10 000 years had elapsed, and assuming that a generation was one year). Although there are many estimates of divergence rate, we

note that $2\% \text{ Ma}^{-1}$ is at the low end (Cyt b, Weir and Schluter 2008), and if higher rates were used, coalescence would be more rapid; hence 2% is a conservative value. We used a gamma rate parameter of 0.7, with four rate categories, and a transition:transversion ratio of 15:1. We recognize that actual values of these parameters differ for each species and gene region. However, these values are approximate to the ones observed in actual sequence data, and we felt they were appropriate to provide a heuristic estimate of sequence evolution on the island. The simulated sequences were input into Arlequin (Excoffier et al. 2005) for analysis, and TreeView (Page 2001) was used to visualize trees derived from the simulated sequence data.

Ecological niche modeling

We used ecological niche modeling to characterize coarse-scale intraspecific niche variation among the six species analyzed genetically in this study, and the curve-billed thrasher, which was previously analyzed morphologically and genetically (Rojas-Soto 2003, Rojas-Soto et al. 2007). Ecological niche models (ENMs) were developed using the genetic algorithm for rule-set prediction (GARP, Stockwell and Peters 1999, Anderson et al. 2002, Graham et al. 2004) based on primary point-occurrence information (Peterson et al. 2002) from the Pacific Plain and the Sonoran Desert localities which included at least 80 locality records per species, that we drew from the 'Atlas of Mexican Bird Distributions database' (Navarro et al. 2003). We used 30'' resolution ($\sim 1 \text{ km}^2$ cell size) and interpolated climate data summarizing 19 bioclimatic variables from the WorldClim project (Hijmans et al. 2005), in combination with elevation, slope and the compound topographic index from the Hydro-1K dataset (USGS 2001). We tested whether these models retained their predictive power when applied to Tiburón Island's endemics. High predictability of a species' occurrence on Tiburón Island would indicate minimal- or no-niche differentiation, whereas low cross-area predictability is consistent with (though not conclusive proof of) intraspecific niche evolution (Peterson and Holt 2003). Following Anderson (2003), we summed the 10 'best' models in a GIS to produce a single consensus map. Geographical niche differentiation was inferred based on the ability of continental populations to predict both specific collecting localities and general areas of Tiburón Island (percentage of predictability).

For visual purposes, ecological niche space for the mainland and island populations was represented by the combination of annual mean precipitation and mean temperature of the wettest quarter. The first variable was chosen on the basis of the importance of humidity on bird plumage coloration (Hubbard 1973) and the second one was chosen because it was the most important environmental variable detected through a principal component analysis (unpubl.) based on the modeling results previously obtained. We graphed the combined values of these two variables for each pixel where they were predicted to occur.

Results

MtDNA phylogeography

None of the six species analyzed showed a pattern of reciprocal monophyly between Tiburón Island and the mainland. For Gambel's quail, the birds from Tiburón Island and two from New Mexico shared the same ND2 sequence. For Cyt b, the five birds from Tiburón Island were compared to four sequences in GenBank (L08382, DQ485889, AFO28759, AFO28762). Although not all individuals were complete for the gene, there was only one variable position in Cyt b for the island birds which defined two haplotypes. A bird from Tiburón Island and the mainland shared a haplotype. Thus, there is very low variation in the quail, and no evidence for a strong differentiation.

Comparisons of 20 ND2 sequences of gila woodpecker (Fig. 2) from Tiburón Island, Baja California, Baja California Sur and Sonora revealed five haplotypes. The two haplotypes from Tiburón Island are intermixed with those from throughout the range excluding those from Baja California Sur, which appear to be segregated from the rest of the samples. The F_{st} value contrasting birds from Tiburón Island and the mainland (excluding southern Baja California) was 0.17 but not significant ($p=0.10$).

For the cactus wren, previous mtDNA analyses (Zink et al. 2001) revealed the existence of two major haplotype groups, one south of 30°N in Baja California, and the other

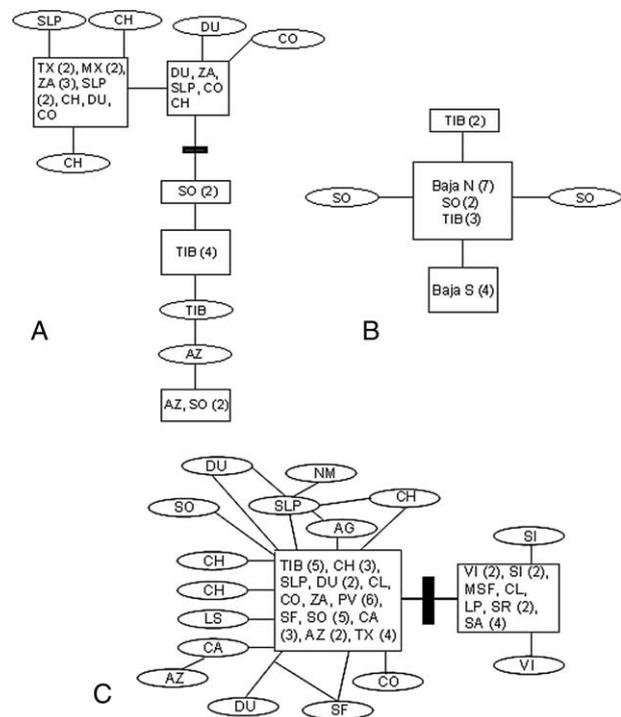


Figure 2. Parsimony networks produced with TCS software (Clement et al. 2000) for (A) ND2 sequences of canyon towhee, (B) partial ND2 sequences for gila woodpecker, and (C) partial control region sequences for cactus wren. Locality abbreviations are given in Fig. 1. Black bars represent major phylogeographic divisions. The number of birds from each locality is indicated in parentheses.

encompassing the rest of the range. Our five samples from Tiburón Island represented a single haplotype that was shared with 26 individuals from most of the sites in the continental clade (CH, SLP, TX, CA, DU, CL, SO, ZA, PV, SF, AZ; see map in Zink et al. 2001). Thus, the Tiburón Island Cactus Wrens are not differentiated from the mainland, and are derived from the main continental clade (Fig. 2). The F_{st} value contrasting birds from Tiburón Island and the mainland (excluding southern Baja California) was 0.005 ($p > 0.5$).

Previous mtDNA control region analyses for Verdin (Zink et al. 2001) revealed the existence of two major haplotype groups, with the same distribution as that described above for the cactus wren. We found two haplotypes among the three individuals sequenced from Tiburón Island, which, like the cactus wren, were scattered among the haplotypes from the main part of the range exclusive of southern Baja California and Baja California Sur (Fig. 2). One haplotype was shared with individuals from many continental localities, whereas the other (differing by 1 substitution) possessed by two birds was unique to Tiburón Island. The F_{st} value of the contrasting birds from Tiburón Island and the mainland (excluding southern Baja California) was 0.42 ($p < 0.001$).

Zink et al. (2001) reported a lack of phylogeographic structure over the range of black-tailed gnatcatcher in spite of a potential east–west distributional gap. The three individuals sequenced from Tiburón Island represented two haplotypes and did not form a clade on the tree or network (unpubl.), which showed numerous loops. One of the haplotypes was found nowhere else, whereas the other was shared with a bird from Coahuila; F_{st} contrasting birds from Tiburón Island and the rest of the range was -0.07 ($p = 0.95$).

The mtDNA gene tree for the canyon towhee shows a division between the Sonoran Desert and the rest of the range (Zink et al. 2001). Our five samples from Tiburón Island represented two haplotypes unique to the island, one shared by four of the five birds. The F_{st} value contrasting samples from Tiburón Island and the Sonoran Desert clade was 0.32 ($p = 0.05$), indicating that the populations differ. The parsimony network (Fig. 2) shows that the haplotypes from Tiburón Island fall within the Sonoran clade. In contrast to the network, a rooted neighbor-joining tree (unpubl.) shows the Tiburón Island birds as a clade. A phylogeographic analysis of the curve-billed thrasher (Rojas-Soto et al. 2007) showed no genetic division between the endemic Tiburón Island population and the mainland ($F_{st} = 0.001$, $p = 0.99$), but the island population is morphometrically divergent (Rojas-Soto 2003).

Simulations

Given the assumptions we made, our simulations showed that if a population on Tiburón Island was isolated from the mainland for 10 000 years, one would expect on average a significant F_{st} value of 0.73 (range 0.45–0.92, Fig. 3); the F_{st} -values for canyon towhee and verdin nearly attained this level. In all trees derived from the simulated sequences, the five individuals from Tiburón were monophyletic, and were

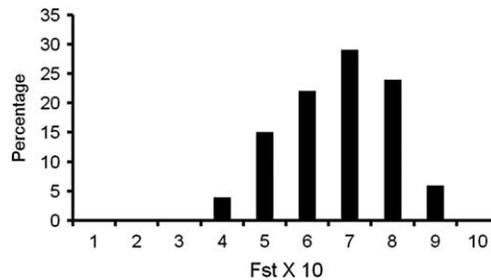


Figure 3. Distribution of simulated F_{st} values, all of which exceed the empirical estimates for the species studied here.

either sister to the mainland haplotypes or were embedded within them.

Ecological niche modeling

The ENM showed two different geographical patterns, one corresponding to those species for which continental populations ‘predict’ the occurrence of 100% of Tiburón Island populations (verdín, gila woodpecker, cactus wren, Gambel’s quail and black-tailed gnatcatcher, Fig. 4a). The second pattern is represented by the curve-billed thrasher (Fig. 4b) and the canyon towhee (Fig. 4c), where none of the island localities were predicted (0% of predictability) and just 4.5% (73 of 1627 pixels) and 1% (17 of 1627 pixels) of the island’s area was predicted, respectively. Thus, the first pattern (represented by five species) is consistent with niche conservatism on Tiburón Island, whereas for the canyon towhee and curve-billed thrasher, niche evolution has occurred. It appears that this niche evolution involves differences in temperature and rainfall (Fig. 5).

Discussion

The relationship among plumage coloration, mtDNA differentiation and ecological niche divergence was inconsistent across species (Table 1). For example, the cactus wren on Tiburón Island is pallid and ash-gray compared to mainland forms, but we found neither mtDNA nor ecological niche differences. In contrast, the canyon towhee is paler and there were substantial mtDNA and niche differences. It is likely that the geographic distance between the island towhees and the nearest mainland population enhances genetic divergence. Thus, the gray coloration can develop both with and without detectable ecological and mtDNA differences.

Our simulations showed that if current-sized populations of our study species had been isolated on Tiburón Island for 10 000 years, haplotype trees ought to recover monophyletic island populations, and F_{st} values should be uniformly large and significant (Fig. 3). If we had used smaller estimates of N_e , then the island population would likely have been reciprocally monophyletic in most or all simulated data sets. Although lack of mtDNA differentiation might sometimes be attributable to human movement of birds, we are unaware of whether local people have transported birds to Tiburón Island, such as quail that have

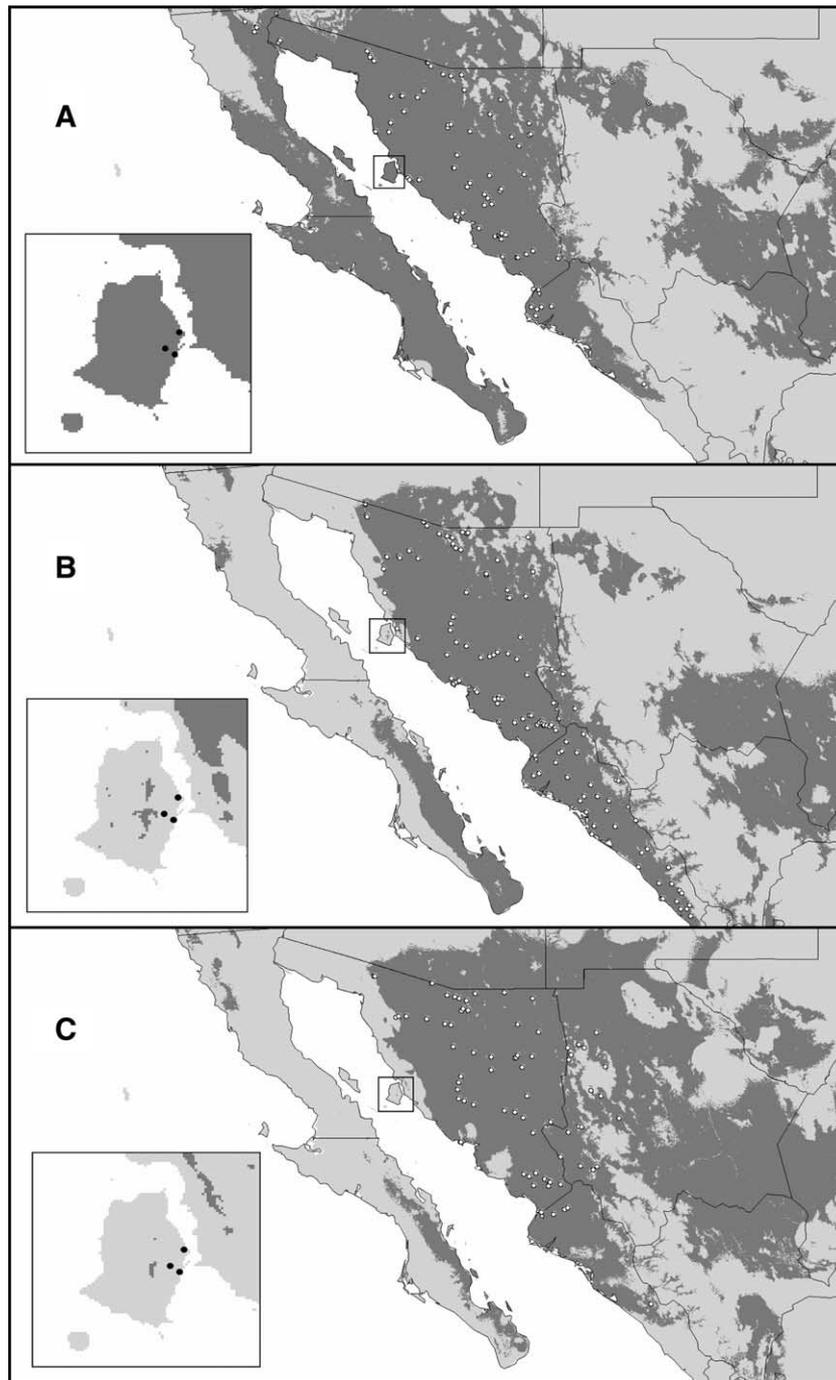


Figure 4. Modeled geographic distribution for: Gambel's quail (A), curve-billed thrasher (B), and canyon towhee (C). Potential presence is represented in dark gray and absence in light gray; white dots represent the locality records used for the model's performance. Dark dots are the localities in the island where the species were collected (but not used for modeling).

been introduced to other islands (e.g. *Callipepla douglasii* in Tres Marias, Howell and Webb 1995). The most likely explanation for why our study species, excluding the verdin and canyon towhee, do not fit the simulation predictions is that gene flow has prevented or retarded genetic differentiation. Immigration to Tiburón Island could be accomplished by direct flight or upon habitat contact during episodes of lowered sea levels. Our samples from the island are too small to determine whether gene flow is ongoing, symmetric, or has been episodic.

The most striking aspect of the phenotypes of Tiburón Island subspecies is the pallid ash-gray coloration (of all but the black-tailed gnatcatcher). One might postulate phenotypic drift to explain morphological differentiation but one would not expect parallel morphological responses (i.e. pallid coloration). Van Rossem (1932, p. 128) noted that "My own interpretation of the evidence is that the action of a change of climate has induced the color changes apparent in the endemic Tiburón Island birds...". He also remarked (1932, p. 124) that the island and mainland

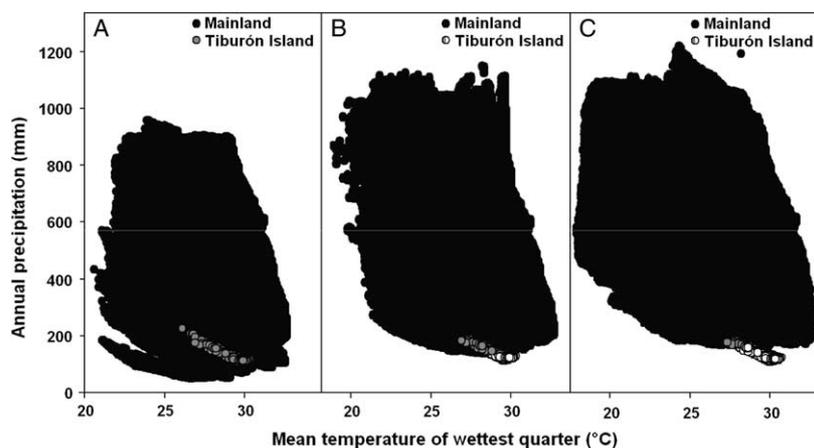


Figure 5. Ecological potential distribution represented by annual mean precipitation and mean temperature of the wettest quarter for: Gambel's quail (A), curve-billed thrasher (B), and canyon towhee (C). Dark dots represent the combination of the two variables per pixel predicted on the mainland (those from Baja California were excluded for better visualization). Light-gray dots represent in (A) the whole predicted island's pixels, and (B) and (C) the non-predicted pixels for Tiburón Island (the combination of the two variables overlaps with mainland conditions). White dots represent the non-predicted pixels for Tiburón Island, which are outside of the mainland conditions.

possess “essentially the same type of soil.” Our models suggested that four of the subspecies that are described as pale and grayer, and the one described as browner, occupy the same environmental space as on the mainland (Fig. 4, 5). Assuming that ecological characteristics of Tiburón Island caused the parallel occurrence of pale coloration (or the browner coloration of the black-tailed gnatcatcher), they were too subtle to be captured by our niche models. However, the two species (canyon towhee and curve-billed thrasher) with significant niche shifts also are pale in coloration. In these two species, niche differences (Fig. 4b–c) are due at least in part to the temperature and rainfall characteristics of the island (Fig. 5b–c). Why the genetically undifferentiated pale gray species did not show differences in these characteristics is unclear (Fig. 4a). Nonetheless, the island's ecology apparently influences the vegetation. Van Rossem (1932, p. 124) remarked “Unquestionably Tiburón possesses a less luxuriant, a less varied, and a more typically Sonoran flora than does the opposite mainland . . .”. Although we have not discovered the exact reasons why the pallid ash-gray coloration is favored, there are qualitative (van Rossem 1932) and quantitative (Fig. 4, 5) hints that rainfall, seasonality and the nature of the vegetation are involved.

Peterson et al. (1999) and Peterson and Holt (2003) concluded that niche conservatism is the dominant theme in bird speciation. That is, allopatric sister species, which differ morphologically, behaviorally and genetically, often occupy very similar niches. In the early stages of phenotypic divergence of Tiburón Island endemics, we also observed niche conservatism, although it is possible our models were not sufficiently sensitive to describe completely the fundamental species' niche (sensu Soberón 2007). However, in two other taxa we documented niche evolution in the initial stages of differentiation. Thus, not all incipient avian divergences occur in the absence of niche evolution.

The observation that parallel morphological responses appear to develop despite gene flow and minor ecological differences provides an indication that the initial stages of divergence of avian populations can occur rapidly, without

genetic isolation, and can be driven by natural selection. As on other islands (Grant 2001), plumage differences might be among the first signs of island diversification. If it were not for the island distinctiveness, these plumage differences might be expressed as clinal differences in a continental setting, and we would observe the typical lack of mtDNA differentiation (Zink 2004).

Relatively few molecular studies in the region provide contrasts to the avian phylogeographic patterns we documented. Radtkey et al. (1997) found five major mtDNA clades for the western whiptail lizards *Cnemidophorus tigris*, and the population from Tiburón Island was placed within the same clade of other northern Sonora and Baja California populations including the deep water island populations (e.g. San Esteban, San Pedro Mártir, San Lorenzo, and Salsipuedes). Although land bridge island populations generally had a sister-species relationship with a nearby mainland population (Radtkey et al. 1997, Upton and Murphy 1997, Ricklefs and Bermingham 2007), Hafner et al. (2001) suggested that insular populations of the white-footed mice *Peromyscus* do not always derive from the adjacent mainland. The situation on Tiburón Island contrasts with other offshore island systems, such as the Alexander Archipelago (Cook et al. 2001) and the Queen Charlotte Islands (Haida Gwaii, Topp and Winker 2008), where most species exhibit genetic differences.

Conservation considerations

For most of described subspecies of birds in Tiburón Island, we find no mtDNA or ecological support. The subspecies without mtDNA differentiation might or might not represent distinct evolutionary entities, depending on the genetic basis of the plumage differences. Based on the degree of genetic differentiation, the verdin and canyon towhee are the only candidates one might consider as a neo-endemic forms (sensu Myers and Giller 1988), and therefore, as evolutionary significant units for conservation purposes (Moritz 1994). However, because the rest of the island subspecies possess some degree of color differentiation, they

might require protection so as to maintain the evolutionary potential of the insular biota. It would be useful to study the island's other resident species to see if there are tendencies towards pale coloration (that did not capture the attention of subspecies taxonomists).

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