 doi: 10.1111/mec.12236

Cryptic introductions and the interpretation of island biodiversity

JULIAN D. AVERY,* DINA M. FONSECA,† PASCAL CAMPAGNE* and JULIE L. LOCKWOOD*
Department of Ecology, Evolution, and Natural Resources, Rutgers University, 14 College Farm Road, New Brunswick, NJ 08902, USA, †Center for Vector Biology, Rutgers University, 180 Jones Avenue, New Brunswick, NJ 08901, USA

Abstract

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

Keywords: Bermuda, cryptogenic, eastern bluebird, island, microsatellite, non-native

Received 23 February 2012; revision received 15 December 2012; accepted 18 December 2012

Introduction

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

A standard metric used in island biogeography is the number of taxa present on one or more islands (Macarthur & Wilson 1967). For the vast majority of these islands, systematists working before the advent of molecular methods determined the taxonomic identities of these species based on morphology alone. There is no doubt that some cryptic species remain to be 'discovered' through the use of molecular and morphological methods, and this certainly will alter components of island biogeography (e.g. Lohman *et al.* 2010). We suggest that it is also likely that some of these taxa

Correspondence: Julian D. Avery, Fax: +1 732 932 8746;
E-mail: juliandavery@gmail.com

represent populations that colonized an island via the direct or indirect action of humans and are thus cryptic invaders.

Species that have become established since modern records (post-1800s) are routinely identified as non-natives, and such species are not considered in most island biogeographical studies. In contrast, cryptic invaders can become established as far back as 1000 years ago, and they are often naively initially identified as natives (Carlton 1996). Mistakenly attributing native status to such species may artificially inflate estimates of island biodiversity. This issue comes to the fore in instances where cryptic invaders evolved substantial life history or morphological features since their time of founding. Is the presence of these species consistent with the theories embodied in island biogeography, or are they something wholly unique that require us to reconsider some of our basic assumptions about the origin and maintenance of island biodiversity? We provide evidence that the eastern bluebird population on the island of Bermuda sits precisely in this grey area, and we describe how the presence of such species (more broadly) challenges our existing framework for understanding island biodiversity.

History of Bermuda and its songbirds

Bermuda (56 km^2) lies in the North Atlantic Ocean with the nearest landfall just over 1000 km south-southeast at Cape Hatteras, North Carolina, USA (Fig. 1) (Olson *et al.* 2005). The island is situated at a strategic location in the Atlantic, which made it an occasional replenishment spot for Spanish and Portuguese ships that sailed this stretch of ocean in the 1500s, and later rendered it a key settlement outpost for the British in the early 1600s (Craven 1937). Unlike many other oceanic islands, Bermuda does not seem to have been inhabited by humans before the first permanent British settlements in 1609 (Verrill 1902). It was not long after the establishment of these early settlements, however, that the flora and fauna began to undergo permanent alteration. The purposeful and accidental importation of plants and animals began almost immediately after initial settlement, with several of these species quickly attaining pest status (Verrill 1902). The British settlers began at once to cut the abundant Bermuda cedar (*Juniperus bermudiana*) and other native trees for the construction of dwellings and fortifications, largely deforesting the island within a span of a few decades (Verrill 1902).

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

The three passerines currently considered native are the eastern bluebird, gray catbird (*Dumetella carolinensis*) and white-eyed vireo (*Vireo griseus*), all of which are widespread across the island. The Bermuda bluebird population was ascribed subspecies status as early as 1901 (Verrill 1901a,b; Bradlee *et al.* 1931; Phillips 1991; Gowaty & Plissner 1998), based upon general impressions of size and colour. These differences in plumage coloration are substantial as confirmed using modern methods of colour measurement (J.D. Avery, P. Cassey, J.L. Lockwood, in Review, below). It is thus understandable that early biologists would assume bluebirds had been evolving in isolation on Bermuda for thousands of years. However, there are no records, going back 400 000 years, of bluebirds as fossils (Olson *et al.* 2005), and they are not explicitly mentioned in the accounts of the birds given by early settlers (Verrill 1902). Bluebirds are native to North America and have no breeding populations on oceanic islands other than Bermuda, including near-shore islands in close proximity to North America (Gowaty & Plissner 1998).

Colonization scenarios

Our primary objective is to determine how long eastern bluebirds have been present in Bermuda. We further investigate whether the Bermuda bluebird population is connected to mainland populations via gene flow. To systematically differentiate these possibilities, we recognized four partially nested colonization scenarios (Fig. 2). At the primary level, and based upon the history of human occupation of Bermuda, we defined two alternate time periods when eastern bluebirds may have become established on Bermuda. Bluebirds were either present before human settlement of the island in 1612 (*presettlement scenario*), or they arrived sometime after 1612 facilitated to some degree by human actions (*post-settlement scenario*). Given the general uptick in numbers of species introduced as exotics to Bermuda after 1612, and the very early known introduction of the northern cardinal, it is possible that eastern bluebirds were brought to Bermuda in the 1600s and released there by early settlers. Also plausible is that the habitat changes

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

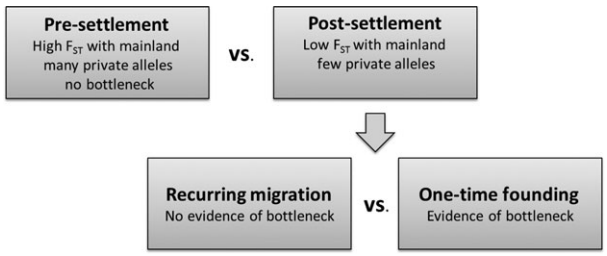

Fig. 2 Schematic of colonization scenarios and resultant expected signatures in genetic diversity. Based on historical and fossil records, the colonization of the eastern bluebird on Bermuda could have occurred since the settlement of the island by the British in the 1600s (postsettlement). On the other hand, the distinct plumage of Bermuda bluebirds has led taxonomists to classify the population as a subspecies thus indicating a much longer tenure of residence on the island (presettlement). If the Bermuda bluebird population colonized the island more recently, we can further explore the extent to which this population has been connected to the North American mainland through migration.

wrought by early human settlers transformed the forested landscape of the island into a state that was highly favourable to bluebirds, being sparsely forested with substantial open ground. Eastern bluebirds are considered facultative migrants, and individuals from the mainland regularly make landfall on the island during their Autumnal migration south (Bradlee *et al.* 1931). Some fraction of these migrating individuals may have remained on the island to breed after the creation of suitable habitat, and thus, they provided the founding individuals for the Bermuda population.

At the second level, we recognized two possibilities for continued gene flow under the *postsettlement scenario* (Fig. 2). We should expect relatively high gene flow if, at some regular interval since the population was initially founded, a fraction of migratory individuals choose to stay on the island and breed (*recurring migration*). Like other oceanic islands, Bermuda hosts a wide array of migratory species each year, including bluebirds, and is thus capable of receiving new immigrants on a regular basis. On the other hand, gene flow could be quite restricted, or non-existent (*one-time founding*), if either (i) after initial colonization of the island, migratory individuals did not continue to regularly stay and breed, or (ii) the founding population was not derived from migrants but was instead derived from individual birds purposefully brought to the island by humans and released there.

Each of these scenarios will leave a genetic imprint on the Bermuda bluebird population, and our goal here is to create an evidence base to test the likelihood of each (Fig. 2). Bermudan populations of eastern bluebirds do not differ from mainland populations in mitochondrial DNA (R. Fleischer, personal communication), so we evaluated our scenarios using microsatellite markers, which are supposed to mutate at a higher rate than mitochondrial DNA and can uncover evidence of

more recent divergence (Fig. 2) (Avise 2004). We expect high genetic differentiation between Bermuda and the mainland populations, many private alleles, and no sign of a recent bottleneck under the *presettlement scenario*. We expect low differentiation and few private alleles under the *postsettlement scenario*. To distinguish between the two scenarios nested under *postsettlement*, we use evidence of a detectable bottleneck. A detectable population bottleneck signature is more likely in the microsatellite information from Bermuda bluebirds under the *one-time founding scenario*, and less likely under the *recurring migration scenario*, although we understand that this can also be influenced by the founding population size (Clegg 2010). Note that given the nested nature of our scenarios, we expect each of these latter scenarios to share with the *postsettlement scenario* the signatures of low genetic differentiation from the mainland and few private alleles.

Methods

Sample collection

We examine eastern bluebirds from Bermuda and from a swath of the nominate race's (*S. s. sialis*) native range in North America (i.e. New Jersey, North Carolina, Iowa and Minnesota; Fig. 1). No matter the colonization scenario, the founding population is likely to have been located along the eastern seaboard or in the northern latitudes of North America, given early shipping and commerce routes and regional climate patterns that drive migratory behaviour. Bluebirds are sedentary to the south and migratory to the north during winter, with birds from the upper Midwest migrating towards the South east United States (Gowaty & Plissner 1998).

We included a total of 114 bluebirds in the analysis from Bermuda and mainland North America (Fig. 1) and initially treated all mainland sample localities as separate populations (Fig. 1). Because of the proximity between individuals caught in New Jersey and North Carolina, as well as between Iowa and Minnesota, we first tested for differences in allele frequencies between each pair of localities. The tests performed on the subdivided data did not identify barriers to gene flow between mainland populations (see below); therefore, we lumped North Carolina and New Jersey together into one population (coastal) and Iowa and Minnesota into another population (continental). We decided this represents a biologically relevant grouping due to the geographic distance involved and evidence of separate migratory pathways between coastal and continental groups gathered from individual bird band recoveries (Gowaty & Plissner 1998).

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

Molecular methods

We washed (0.5% SDS and TE) and extracted genomic DNA using the manufacturer's room temperature–pH treatment protocol. We genotyped all individuals at 12 microsatellite loci (Sialia2, Sialia6, Sialia8, Sialia11, Sialia15, Sialia18, Sialia22, Sialia27, Sialia28, Sialia30, Sialia36 and Sialia37) (Faircloth *et al.* 2006) following the PCR protocol developed by Faircloth *et al.* (2006). PCR amplifications were performed in 20 μL volumes. Our thermal touchdown cycle (following the 60–49.5 °C program) differed from the study by Faircloth *et al.* (2006) in the following parameters: 95 °C for 5 min at beginning of routine; 95 °C for 30 s at start of each cycle; 30 s at the highest annealing temperature −0.5 °C per cycle; 72 °C for 30 s for a total of 21 cycles; followed by nine cycles of 95 °C for 30 s; 49.5 °C for 30 s; and 72 °C for 30 s. We visualized PCR products on 1% agarose gels and adjusted the number of extension cycles for different primer pairs to control the overall quantity of product generated for each primer set. All loci were subjected to a routine of 21 and nine cycles except for Sialia11, Sialia18, Sialia28 and Sialia36, which we subjected to 21 and 10 cycles. Sialia15 underwent a routine of 21 and eight cycles. We multiplexed PCRs differentiating between overlapping loci with fluorescent dyes (PET, VIC, FAM), and we manually scored genotypes using Genemapper version 3.7 (Applied Biosystems, Foster City, CA).

Statistical methods

We calculated summary statistics and population differentiation (AMOVA) using GENALEX 6.3 (Peakall & Smouse 2006), F_{STAT} (Goudet 1995) and ARLEQUIN 3.11 (Excoffier *et al.* 2005). To account for the effects of genetic variation on F_{ST} values, we applied Meirmans correction (F_{STc}) (Meirmans 2006) and calculated D_{est} (Jost 2008) using SMOGD (Crawford 2010). We used Genepop 4.0 (Rousset 2008) to test for linkage disequilibrium and deviations from Hardy–Weinberg (using the parameters 10 000 dememorizations; 100 batches; 5000 iterations per batch) and applied Bonferroni corrections to control for type I error. We implemented STRUCTURE 2.3.3 (Pritchard *et al.* 2000) as another tool to detect population differentiation setting the number of

clusters (K) from 1 to 5 with 10 iterations for each K and a burn-in period of 10^4 and 10^5 Markov chain Monte Carlo repetitions. We used both the admixture and no-admixture ancestry models with sampling locations as priors when previous runs failed to detect structure in our data. We used program BOTTLENECK (Piry *et al.* 1999), M P Val (Garza & Williamson 2001) and a graphical method devised by Luikart *et al.* (1998) to test for a recent population bottleneck in the Bermuda bluebird population. We ran all three models in BOTTLENECK because our data appear to have some loci conforming to the infinite and stepwise mutation models, and it is not clear given the information at hand which method is most appropriate (Darvill *et al.* 2010). To test for differences in allelic diversity, we ran a one-way ANOVA using SAS software (SAS Inst. Cary, NC, USA).

Founding simulation

To help interpret our empirical data set in light of our colonization scenarios, we designed a simulation that mimicked the founding of an island population by mainland individuals and then tracked the resultant microsatellite allele frequencies (Fig. 3). By varying elements of this model (see below), we generated a suite of possible genetic indices within the island population. Those indices that diverged greatly from our observed indices suggest that the underlying mechanisms that produced them can be discounted as driving our empirical results. By analogy, those simulated scenarios that produce indices very much like our observed results can be considered the more likely mechanisms. We provide a full description of our simulation according to the ODD-protocol (Grimm *et al.* 2006, 2010) in the Supporting information and only briefly describe its structure here and in Fig. 3.

We explored the influence on allele frequencies of various migration rates, founding population sizes, residence times, carrying capacities, mutation rates and growth rates of the island population. The first three of these parameters differentiate among our four colonization scenarios (Fig. 2). The time of residence was set at either 1000 or 200 generations, where the former reflects the *presettlement scenario* and the latter the *postsettlement scenarios*. Migration rate was allowed to vary substantially reflecting our uncertainty surrounding the degree to which gene flow has influenced the genetic makeup of the Bermuda bluebird population and allowing us to differentiate between the *recurring migration* and *one-time founding scenarios*. The founding populations we explored were all relatively small ($\leq$100 individuals) reflecting our supposition that the Bermuda bluebird population either self-colonized via migratory individuals or was purposefully introduced by early human settlers.

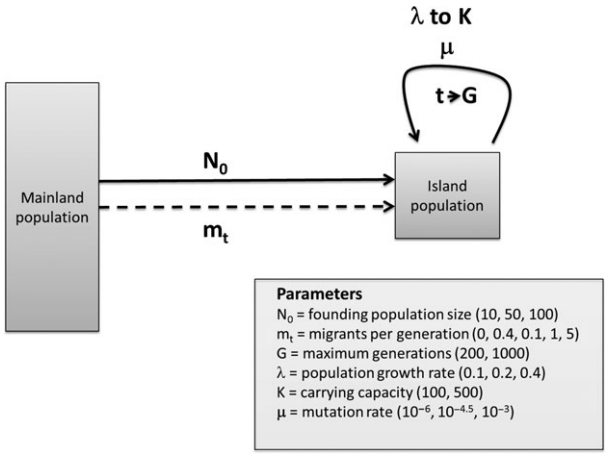

Fig. 3 A schematic of our founding simulation, defining associated parameters and the values they can take. Each simulation was initiated at generation $t = 0$, where a set of N_0 individuals is introduced to the island. Their genotype was randomly drawn from the empirically observed frequencies of alleles in our pooled set of North American bluebird samples. Simulated migration is allowed at any generation (t), where m_t individuals migrate from the mainland to the island (reciprocal exchange is ignored). The genotypes of these migrants are randomly drawn from the mainland allele pool. These founding individuals randomly mate, and the genotype of the progeny is randomly determined in accordance with Mendellian rules. The sex of the progeny is randomly assigned with a probability of 0.5, and number of young produced per mating varies according to published bluebird clutch sizes. Heritable mutations may occur randomly in the genotype of any progeny at a rate μ (per individual and per generation). The position of the mutation in the genotype of mutants is randomly assigned (any loci, any alleles). This process continues until G the total number of generations is reached. Island population size is never allowed to surpass carrying capacity (K), but grows to carrying capacity from small founding sizes according to a constant rate λ.

The last three parameters were varied in accordance with our uncertainty in their true values within the Bermuda bluebird population. By allowing these parameters to vary, we gained insight into the sensitivity of our results to underlying demographic uncertainty. Carrying capacity was set to reflect the range of empirical estimates for the current size of the Bermuda bluebird population (D. Wingate, personal communication). The population growth rate assumed one of three values based on the evidence in Gowaty & Plissner (1998). The mutation rate varied over a reasonable range for microsatellites (Avise 2004; Ellegren 2004; McConnell *et al.* 2007).

In each run of the founding simulation, we systematically combined all possible combinations of the above six parameters to produce 540 ending island allele frequencies. After the maximum number of generations was reached, individuals were sampled from both the

mainland and the island populations, respectively, to constitute a genetic data set whose structure (number of loci, number of individuals) was identical to our observed data set. From this simulated genetic data set, we calculated the same set of nine indices we did for our observed data above (Table S5, Supporting information). Each 'run' thus created a matrix of nine genetic indices for 540 simulated island populations. The similarity between the observed data and the simulations was assessed by Euclidean distances computed on independent variables. We did this by performing a centred and standardized principal component analysis upon the 540 × 9 matrix. The values in this matrix were centred upon the values generated by our observed bluebird data set. Next, we calculated the Euclidean distances of the 540 simulated island populations to the centre of the PCA (corresponding to the observed data) and noted the starting parameters for the one simulated population that most closely matched our observed data. We repeated the above process (a 'run') 3149 times for a total of 1 700 460 simulated island populations. By retaining the simulation that most closely approximated our empirical data in each run, we were left with a total of 3149 simulations that could be used to identify which starting parameters resulted in the most similar genetic indices to our observed indices.

Results

The numbers of alleles ranged from 4 to 20 per locus, and all loci were polymorphic in all populations except for Sialia18, which was monomorphic in Bermuda (Table S1, Supporting information). Overall allelic richness was significantly lower in the Bermuda bluebird population, despite a greater sample size than either the coastal or continental mainland populations (avg. richness per locus in Bermuda and Mainland = 3.90 and 8.30, respectively) (Table S1, Supporting information). Bermuda bluebirds possessed two novel alleles with a frequency of 0.098 (eight heterozygote individuals with a single copy) and 0.012 (one heterozygote individual with a single copy). Private alleles were never found in more than three individuals on the mainland.

Linkage disequilibrium and Hardy–Weinberg equilibrium

We found evidence for linkage disequilibrium between two pairs of loci after Bonferroni correction ($P_{Bonferroni} < 0.0007$). Loci Sialia36 and Sialia8 ($P = 0.00005$, SE = 0.000044, switches = 25 049), as well as Sialia37 and Sialia8 ($P = 0.000382$, SE = 0.000204, switches = 31 568), were out of equilibrium only in the Bermuda population and did not show linkage disequilibrium in the North American populations for the same loci as described in Faircloth *et al.* (2006). Thus, we suspect they are not physically linked. We found two loci that did not conform to Hardy–Weinberg equilibrium after Bonferroni correction: Sialia27 in the Coastal population and Sialia36 in the Bermuda population (Table S2, Supporting information). Sialia36 exhibited a heterozygote excess, and Sialia27 exhibited a homozygote excess. Removing these loci from subsequent analyses did not change the overall results with one exception (see below); therefore, we retained them in the data set. When we analysed each individual sampling locality, the deviation from Hardy–Weinberg in Sialia 27 appears to be driven by the North Carolina samples.

Population divergence

Our F_{ST} and D_{est} values clearly show divergent allele frequencies in the Bermuda bluebird population relative to the mainland populations (Table S3, Supporting information) ($F_{2,114} = 8.48$, $P = 0.010$). Standardized F_{STc} values show that allele frequencies in Bermuda are approximately 32% divergent from the mainland (Table S3, Supporting information). There is no evidence for divergence between mainland populations. Our STRUCTURE results consistently showed support for an island cluster and a mainland cluster. Choosing the no-admixture model with prior location information did not help to resolve sampling localities on the mainland (in ungrouped and grouped form) and failed to detect any individuals that clustered outside their original mainland or island sampling region (Fig. S1, Supporting information).

Prior to lumping the Coastal and Continental populations, when Loci Sialia27 was removed from the data set, we did find a significant difference in allele frequencies between Iowa/Minnesota and North Carolina using the exact *G* test in Genepop. However, we did not see any differences with the data structured into Coastal and Continental groupings when loci not in Hardy–Weinberg were removed. All other tests for population differentiation in Genepop were consistent with the AMOVA results from GENALEX. F_{IS} values, a measure of inbreeding, were low (Bermuda: 0.036, Cont.: 0.033, Coast: 0.034) and not significantly different in each of the three localities based upon a permutational test in ARLEQUIN.

Bottleneck analysis

We found evidence that some loci appear to exhibit stepwise mutation behaviour, while others appear to behave in ways expected under the infinite allele model (IAM); thus, we tested for evidence of a bottleneck under each assumption. Under the IAM, tests were

marginally nonsignificant with the sign test ($P = 0.057$, $n = 82$) and Wilcoxon test ($P = 0.073$, $n = 82$) for heterozygote excess expected under a recent bottleneck scenario. The stepwise mutation model (SMM) and two-phase model (TPM) were not significant ($P = 0.56$ and 0.54, respectively). We also followed the graphical approach devised by Luikart *et al*. (1998), and this evidence indicates a shift in the allele frequency distribution between the Bermuda and North American bluebird populations, a signal that a population bottleneck has occurred within the Bermuda population (Fig. 4). Both methods are thought to only be effective at detecting recent bottlenecks from $2N_e$ to $4N_e$ generations (Luikart *et al*. 1998; Piry *et al*. 1999). Using M P Val (Garza & Williamson 2001), we obtained a critical M value of 0.86, which does not indicate a recent bottleneck. In addition, it is apparent that the range of alleles from the Bermuda population is a subset of the most common alleles from the mainland populations, another indicator of a founding effect (Fig. 5). Finally, there are four loci in which a single Bermudan allele accounts for more than 80% of the frequency. Taken together, this evidence suggests that the Bermudan bluebird population underwent either a moderately narrow genetic bottleneck in the recent past (<100 years) which seems unlikely given our M value, or such a severe bottleneck in the more distant past (>100 years) that its genetic signature is still apparent. It is also possible that rapid population recovery in either case could have preserved some of the rare alleles that would otherwise have been lost, thus influencing the strength of the bottleneck signal (Nei *et al*. 1975).

Founding simulation

Overall, our model performed well and followed expected patterns in population genetics. For instance, an increase in the number of simulated generations only affected genetic structure when migration was limiting (Fig. S2, Supporting information). Furthermore, low founding population sizes reduced the number of polymorphic loci and number of effective alleles as expected (Fig. S2, Supporting information).

With regard to our founding simulations, our model clearly showed support for a relatively recently established population (Fig. 6). Simulations where residence time on the island did not exceed 200 generations provided genetic indices similar to our observed indices in 93% of the replicate runs (Fig. 6). Despite a range of possible mutation and population growth rates, almost no simulations involving residence times of 1000 generations resulted in observations similar to our observed data. When residence time was limited to 200 generations, low or null migration rates (0.1–0.0 per generation) comprised the majority of indices that were most similar to our observed data (Fig. 6). Indices produced with a high migration rate were never similar to our empirical data except when residence time was 1000 generations. We found greater support for a founding population size of 50 individuals rather than 10 or 100.

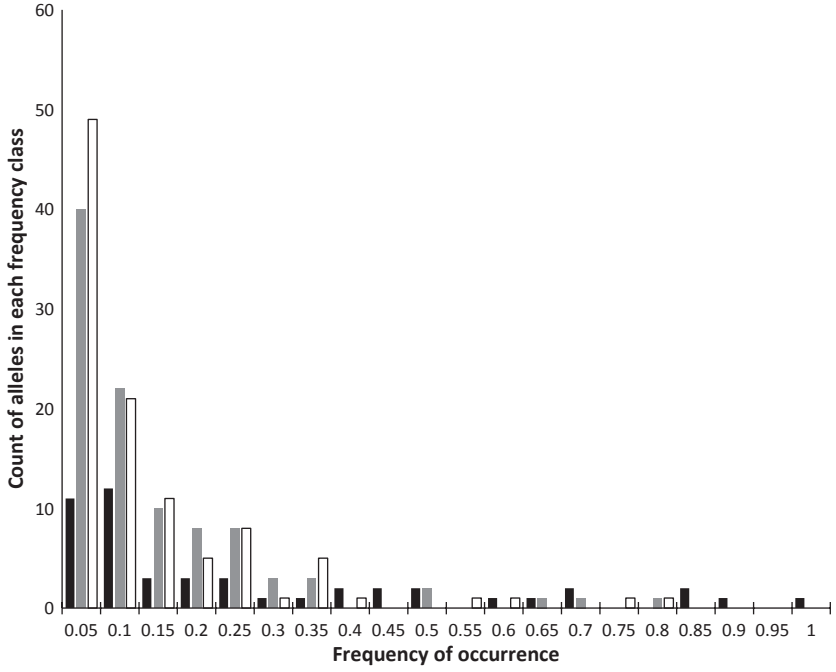

Fig. 4 Total allelic counts in 5% frequency increments for 12 eastern bluebird microsatellite loci sampled from Bermuda (black bars), the inner-continental North American mainland (grey bars) and Atlantic coast of North America (white bars). In nonbottlenecked populations, there are many alleles that occur at low frequencies and few alleles that occur with high frequency. Note the longer tail for Bermuda, indicative of more individual alleles that occur with greater frequency. In Bermuda, one locus was fixed and two more loci had a maximum of two alleles. Bermuda samples show a characteristic hump shape that signifies a shift towards fewer rare alleles, indicative of a genetic bottleneck.

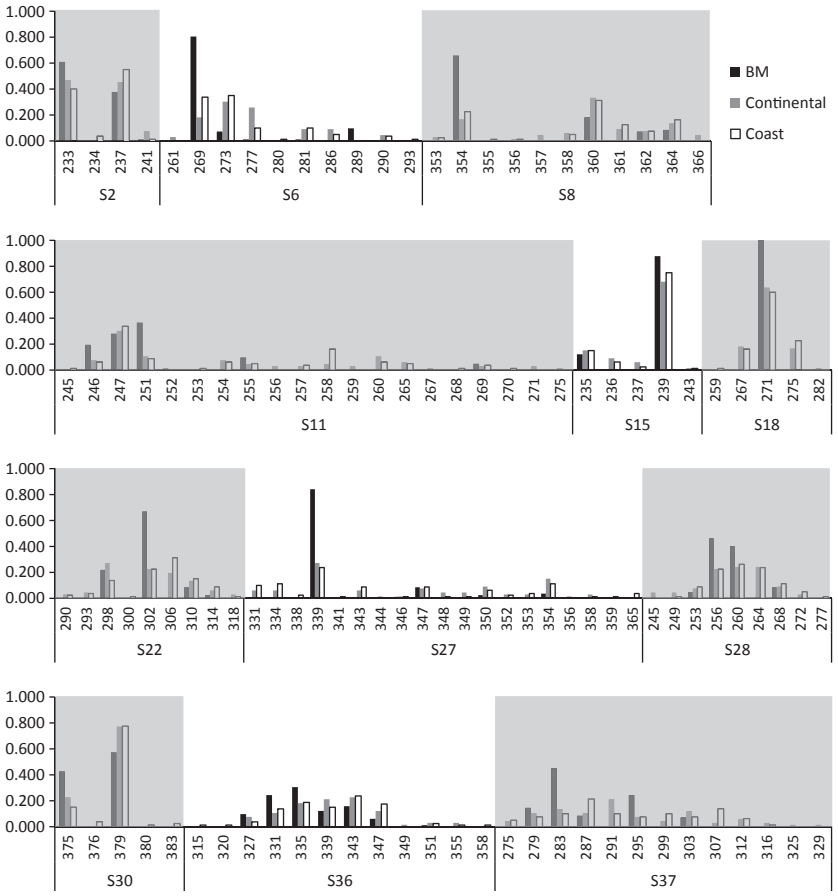

Fig. 5 Allele frequencies across all sampled populations for each locus. Allele number and locus are distributed along the *x*-axis, and percentage is depicted along the *y*-axis. The Bermuda population is comprised of the most common mainland alleles, and the frequencies of these alleles are often much greater in the Bermuda population than in the mainland population.

Varying mutation rates, carrying capacity and population growth rates, did not substantially impact the degree of similarity between the simulated genetic indices and the observed indices (Fig. 6).

Discussion

If the evolution of substantial morphological and life history differences between populations takes considerable time, then it was perfectly reasonable for the intrepid biologist to assume that a distinct form they found on an island was native to that island. There are two observations that challenge this long-held assumption. First, species regularly colonize islands and evolve in contemporary time [less than a few hundred years, (Reznick & Ghalambor 2001; Stockwell *et al.* 2003; Clegg *et al.* 2008)], and the resultant divergence in traits between populations can be quite substantial (Vellend *et al.* 2007). Second, the dynamics of human settlement across the globe involve considerable trade in live species, and in some geographical locations (principally islands), human settlement occurred within the last millennium (Steadman 2006; Fitzpatrick & Keegan 2007; Whittaker & Fernandez-Palacios 2007). Given these observations, the contemporary biologist is confronted with the possibility that the unusual variety of plant or animal she found on an isolated island either is not native to that island or what biologists generally refer to as 'native' has some intriguing and challenging exceptions.

Our analysis of eastern bluebirds across mainland and island populations illustrates the potential problems that scientists may find lurking in island systems. Despite their unique morphology (J.D. Avery, P. Cassey, J.L. Lockwood, in review), bluebirds on the island of Bermuda appear to be a recently isolated population based upon three lines of evidence. First, we found a profound lack of *in situ* genetic variation among the individual bluebirds resident on Bermuda (Phillimore *et al.* 2008; Clegg 2010). While mutation rates for microsatellites are highly variable, published rates suggest that if the Bermuda population had been isolated for thousands of years, we would expect to see considerably more than the two novel alleles currently present in the Bermuda population (Clegg 2010). Our founding simulation supports this conclusion and shows that the observed genetic indices for Bermuda bluebirds are most consistent with a recently isolated population. Second, North American and Bermudan populations show

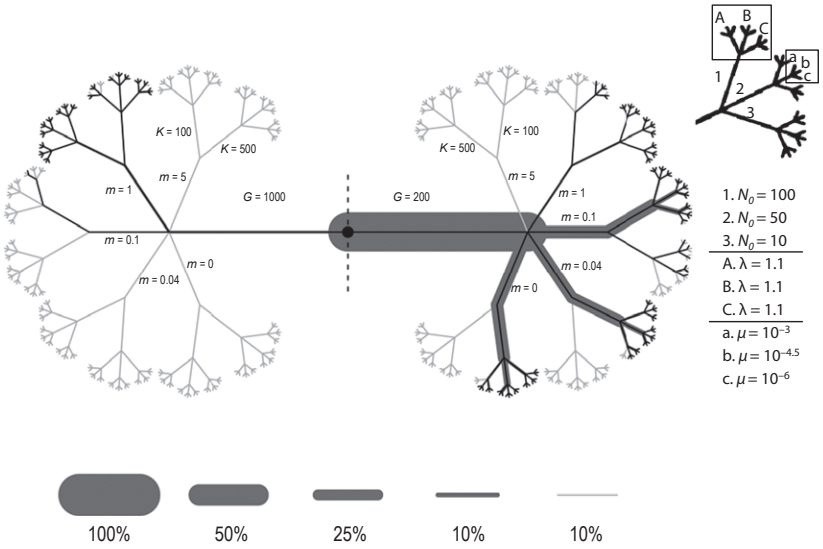

Fig. 6 A dendrogram illustrating the results of our founding simulation whereby we vary six parameters to explore their influence on genetic diversity within an island population founded from a mainland source. Each split in the 'tree' represents a different value for each of the six parameters explored. Parameters follow Fig. 3 and are G = residence time in generations, m = migration rate per generation, K = carrying capacity of the island population, N_0 = founding population size, λ = population growth rate, μ = mutation rate per generation. The more basal the split in the tree, the greater is the importance of this parameter in creating genetic indices similar to our observed indices for Bermuda bluebirds. The weight (in grey scale) of the branches indicates the percentage of model runs that produced indices similar to our observed indices. Parameters are repeated in a clockwise pattern on the right-hand side and counter-clockwise on the left. We broke out a section of the figure to label smaller branches of the tree, and this branch is representative of the other 19 sections.

30% divergence in allele frequencies, which is quite striking in light of the dearth of novel alleles. This observation paired with our simulation results indicates that the likelihood of ongoing gene flow from North American to Bermuda is very low. Third, we observed that common mainland alleles are also the most common island alleles and that single alleles account for a large proportion of the variability at each locus in Bermuda (e.g. allele 269 at Locus Sialia6 has a frequency of 81%) (Mills 2007).

Given our evidence and the absence of bluebirds in the fossil record or early written accounts of Bermuda, we can reasonably reject the *presettlement scenario*. A lack of fossils is not conclusive (Rando *et al.* 2010) but becomes more compelling with our additional data. The question then centres on the manner in which the bluebird colonized Bermuda and to what extent this population maintains a genetic connection to the mainland. We find little support for the *recurring migration scenario* due to the presence of strongly divergent allele frequencies, no evidence from our founding simulation that even moderately high migration rates would produce the observed genetic indices on Bermuda, and a signal of a somewhat persistent population bottleneck. Our evidence also collectively supports a single founding event. Unfortunately, based on our evidence, we cannot differentiate between a one-time founding of individual bluebirds brought to the island by early settlers vs. a one-time founding of migratory individuals that remained on the island to breed.

Nevertheless, our conclusion that the Bermuda bluebird population was founded recently leads to several salient points regarding the evolution of phenotypic differences, in this case plumage colour. J.D. Avery, P. Cassey, J.L. Lockwood (in review) quantitatively established substantial differences in the structural blue coloration of the plumage in both male and female Bermuda bluebirds as compared to North American mainland population. Since its founding, male and female Bermuda bluebird plumage has become brighter than individuals present on the mainland. The plumage of Bermuda bluebirds has also shifted in hue towards longer wavelengths. Finally, Bermuda bluebirds have maintained a high level of sexual dichromatism despite isolation on an island, which is unusual for birds (Price, 2008; J.D. Avery, P. Cassey, J.L. Lockwood, in review). Thus, if the bluebird colonized Bermuda in the 1600s as we show here, it must have evolved these phenotypic differences over this same interval. While we do not know the exact mechanisms influencing the evolution of plumage colour in this system, there is the possibility of either adaptive (e.g., natural or sexual selection) or nonadaptive (e.g. founding event) factors influencing plumage differentiation from the mainland population.

Although postinvasion evolution is certainly common, this would represent one of the most recent instances of a cryptogenic species having accumulated differences to the extent that it could legitimately be considered a subspecies (Johnston & Selander 1964; Vogel *et al.* 2003; Ricklefs & Bermingham 2008).

In light of our results, the subspecies status of Bermuda bluebirds presents an interesting wrinkle in our interpretation of island biodiversity and its generation. Eastern bluebird morphology is clearly evolving at a faster pace than neutral nuclear markers and significantly faster than what is often expected for recent population splits (Stockwell *et al.* 2003; Dlugosch & Parker 2008; Oyler-McCance *et al.* 2010; Pérez-Emán *et al.* 2010; Pruett & Winker 2010). This mismatch between phenotypic and neutral molecular change may be a common by-product of evolution in response to anthropogenic selection pressures (Stockwell *et al.* 2003) or the early stages of colonization (Reznick & Ghalambor 2001), perhaps most commonly for species that are non-native to the region in which they are evolving (Dlugosch & Parker 2008). If the movement of live plants and animals via trade has been part and parcel to the presence of permanent human settlements, there are perhaps many species that are morphologically unique but of relatively recent human-assisted origin (Ricklefs & Bermingham 2008; Grueber & Jamieson 2011). Notably, our results suggest that the other two passerine species currently considered native to Bermuda, the gray catbird and white-eyed vireo, deserve in-depth investigation as to their origins. The white-eyed vireo is also considered an endemic Bermudan subspecies as it shows considerable differentiation in morphology relative to mainland populations and thus may provide further insight into the generation of diversity on the island.

While many researchers are attempting to highlight cryptic diversity in the form of undescribed species in island habitats (see Lohman *et al.* 2010), we feel that revisiting island populations will undoubtedly uncover more situations of contemporary microevolutionary shifts following recent natural colonization or human-facilitated colonization of islands (Reznick & Ghalambor 2001). This will be especially true in cases where there are unexplained gaps in species distributions (Ricklefs & Bermingham 2008), particularly given the short amount of time it took for eastern bluebirds and other species to diverge in morphology (J.D. Avery, P. Cassey, J.L. Lockwood, in review; Clegg *et al.* 2008; Stockwell *et al.* 2003).

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

Acknowledgements

We thank E. Cameron, B. Faircloth, A. Novy, P. Cassey, P. Morin, S. Smith, K. and G. Rossiter, H. Munro, D. Wingate, P. Gowaty, S. Olson, R. Fleischer, J. B. Avery and three anonymous reviewers for their input and help on various parts of this manuscript. We are especially grateful to P. Smouse for many insightful discussions and to Phyllis Greenberg for generous funding and a keen interest in birds. The Bermuda Audubon Society, the Rutgers School of Environmental and Biological Sciences, and the Rutgers Graduate Program in Ecology and Evolution also provided important funding.

References

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*, 2nd edn. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Blakeslee AMH, Byers JE, Lesser MP (2008) Solving cryptogenic histories using host and parasite molecular genetics: the resolution of *Littorina littorea's* North American origin. *Molecular Ecology*, **17**, 3684–3696.

Bradlee TS, Mowbray LL, Eaton WF (1931) A list of birds recorded from the Bermudas. *Proceedings of the Boston Society of Natural History*, **39**, 279–382.

Campbell TW (1995) *Avian Hematology and Cytology*. State University Press, Ames, Iowa.

Carlton JT (1996) Biological invasions and cryptogenic species. *Ecology*, **77**, 1653–1655.

Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. *Science*, **261**, 78–82.

Clegg SM (2010) Evolutionary changes following island colonization in birds: empirical insights into the roles of microevolutionary processes. In: *The Theory of Island Biogeography Revisited* (eds Losos JB, Ricklefs RE), pp. 476. Princeton University Press, Princeton, New Jersey.

Clegg SM, Frentiu FD, Kikkawa J, Tavecchia G, Owens IPF (2008) 4000 years of phenotypic change in an island bird: heterogeneity of selection over three microevolutionary timescales. *Evolution*, **62**, 2393–2410.

Cox GW (2004) *Alien Species and Evolution*. Island Press, Washington.

Craven WF (1937) An introduction to the history of Bermuda. *The William and Mary Quarterly, Second Series*, **17**, 176–215.

Crawford NG (2010) SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources*, **10**, 556–557.

Darvill B, O'Connor S, Lye GC, Waters J, Lepais O, Goulson D (2010) Cryptic differences in dispersal lead to differential sensitivity to habitat fragmentation in two bumblebee species. *Molecular Ecology*, **19**, 53–63.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. *Nature Reviews*, **5**, 435–445.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Faircloth BC, Keller GP, Nairn CJ, Palmer WE, Carroll JP, Gowaty PA (2006) Tetranucleotide microsatellite loci from eastern bluebirds Sialia sialis. *Molecular Ecology Notes*, **6**, 646–649.

Fitzpatrick SM, Keegan WF (2007) Human impacts and adaptations in the Caribbean Islands: an historical ecology approach. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, **98**, 29–45.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.

Goudet J (1995) Fstat version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity*, **86**, 485–486.

Gowaty PA, Plissner JH (1998) Eastern Bluebird (*Sialia sialis*). In: *The Birds of North America Online*, No. 381 (eds Poole A, Gill FB). Cornell Lab of Ornithology, Ithaca, New York, doi: 10.2173/bna.381.

Grimm V, Berger U, Bastiansen F *et al.* (2006) A standard protocol for describing individual-based and agent-based models. *Ecological Modelling*, **198**, 115–126.

Grimm V, Berger U, DeAngelis DL *et al.* (2010) The ODD protocol: a review and first update. *Ecological Modelling*, **221**, 2760–2768.

Grueber CE, Jamieson IG (2011) Low genetic diversity and small population size of Takahe *Porphyrio hochstetteri* on European arrival in New Zealand. *Ibis*, **153**, 384–394.

Johnston RF, Selander RK (1964) House sparrows: rapid evolution of races in North America. *Science*, **144**, 548–550.

Jost L (2008) Gst and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015–4026.

van Leeuwen JFN, Froyd CA, Van der Knapp WO, Coffey EE, Tye A, Willis KJ (2008) Fossil pollen as a guide to conservation in the Galapagos. *Science*, **322**, 1206.

Lockwood JL, Moulton MP (1994) Ecomorphological pattern in Bermuda birds: the influence of competition and implications for nature preserves. *Evolutionary Ecology*, **8**, 53–60.

Lohman DJ, Ingram KK, Prawiradilaga DM *et al.* (2010) Cryptic genetic diversity in "widespread" southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biological Conservation*, **143**, 1885–1890.

Luikart G, Allendorf FW, Cornuet J-M, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.

Macarthur RH, Wilson EO (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, New Jersey.

McConnell R, Middlemist S, Scala C, Strassmann JE, Queller DC (2007) An unusually low microsatellite mutation rate in *Dictyostelium discoideum*, an organism with unusually abundant microsatellites. *Genetics*, **177**, 1499–1507.

McGlashan DJ, Ponniah M, Cassey P, Viard F (2008) Clarifying marine invasions with molecular markers: an illustration based on mtDNA from mistaken calyptraeid gastropod identifications. *Biological Invasions*, **10**, 51–57.

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution*, **60**, 2399–2402.

Mills LS (2007) *Conservation of Wildlife Populations: Demography, Genetics, and Management*. Blackwell, Malden, Massachusetts.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Olson SL, Wingate DB, Hearty PJ, Grady FV (2005) Prodromus of vertebrate paleontology and geochronology of Bermuda. *Monografies de la Societat d'Historia Natural de les Balears*, **12**, 219–232.

Oyler-McCance SJ, John JS, Quinn TW (2010) Rapid evolution in lekking grouse: implications for taxonomic definitions. *Ornithological Monographs*, **67**, 114–122.

Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pérez-Emán JL, Mumme RL, Jabłoński PG (2010) Phylogeography and adaptive plumage evolution in central american subspecies of the slate-throated redstart (*Myioborus miniatus*). *Ornithological Monographs*, **67**, 90–102.

Phillimore AB, Owens IPF, Black RA, Chittock J, Burke T, Clegg SM (2008) Complex patterns of genetic and phenotypic divergence in an island bird and the consequences for delimiting conservation units. *Molecular Ecology*, **17**, 2839–2853.

Phillips AR (1991) *The Known Birds of North and Middle America: Part II*. Published by the author, Denver, Colorado.

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.

Price, T (2008) *Speciation in Birds*. Roberts and Company, Greenwood Village, Colorado.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, **155**, 945–959.

Pruett CL, Winker K (2010) Alaska song sparrows (*Melospiza melodia*) demonstrate that genetic marker and method of analysis matter in subspecies assessments. *Ornithological Monographs*, **67**, 162–170.

Rando JC, Alcover JA, Illera JC (2010) Disentangling ancient interactions: a new extinct passerine provides insights on character displacement among extinct and extant island finches. *PLoS ONE*, **5**, e12956.

Remsen JVJ (2010) Subspecies as a meaningful taxonomic rank in avian classification. *Ornithological Monographs*, **67**, 62–78.

Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112**, 183–198.

Ricklefs RE, Bermingham E (2008) Likely human introduction of the red-legged thrush (*Turdus plumbeus*) to Dominica, West Indies. *Auk*, **125**, 299–303.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences*, USA, **99**, 2445–2449.

Steadman DW (2006) *Extinction and Biogeography of Tropical Pacific Birds*. University of Chicago Press, Chicago, Illinois.

Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, **18**, 94–101.

Vellend M, Harmon LJ, Lockwood JL *et al.* (2007) Effects of exotic species on evolutionary diversification. *Trends in Ecology & Evolution*, **22**, 481–488.

Verrill AH (1901a) Additions to the avifauna of the Bermudas with diagnoses of two new subspecies. *American Journal of Science*, **12**, 64–65.

Verrill AH (1901b) Notes on the birds of the Bermudas with descriptions of two new subspecies and several additions to the fauna. *Osprey*, **5**, 82–85.

Verrill AE (1902) *The Bermuda Islands. An Account of Their Scenery, Climate, Productions, Physiography, Natural History and Geology, with Sketches of Their Discovery and Early History, and the Changes in their Flora and Fauna Due to Man*. Addison E. Verrill, New Haven, Connecticut.

Vogel P, Cosson J-F, Jurado LFL (2003) Taxonomic status and origin of the shrews (Soricidae) from the Canary islands inferred from a mtDNA comparison with the European *Crocidura* species. *Molecular Phylogenetics and Evolution*, **27**, 271–282.

Whittaker RJ, Fernandez-Palacios JM (2007) *Island Biogeography: Ecology, Evolution, and Conservation*, 2nd edn. Oxford University Press, New York.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH (2008) Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *Proceedings of the National Academy of Sciences, USA*, **105**, 7676–7680.

Yan X, Zhenyu L, Gregg WP, Dianmo L (2001) Invasive species in China - an overview. *Biodiversity and Conservation*, **10**, 1317–1341.

J.D.A. designed and carried out the research as well as analysed the data and wrote the article. D.M.F. provided laboratory space and helped analyse the data. P. C. wrote, implemented and interpreted the founding parameter model and helped incorporate the results. J. L.L. helped design the research and write the article. J. D.A. conducts research on contemporary evolution and the processes contributing to biodiversity. D.M.F. studies the introduction, expansion and transformation of invasive species, particularly disease vectors. P.C. studies macro-level processes in population genetics and develops analytical methods to increase the inference of genetic data sets. J.L.L. conducts research on species invasions and extinctions and how they combine to reshape biodiversity.

Data accessibility

A file with data for each individual is archived in DRYAD entry doi:10.5061/dryad.1tv93.

This file includes the individual ID and sampling locality along with the complete genotype at all 12 loci. We have also included a readme.txt file with a detailed explanation of the data archived in DRYAD. To make our data more accessible we have included the original GENALEX and STRUCTURE files along with the associated parameter files that were used in our analyses. We have also archived the R script files used in our founding simulation.

Supporting information

Additional supporting information may be found in the online version of this article.

Data S1 Supporting allelic data and founding materials and methods.

Fig. S1 STRUCTURE results for $K = 3$ populations.

Fig. S2 Effects of individual model parameters upon the following genetic indices; population differentiation (F_{STc}), average number of effective alleles (A_e), and the number of fixed or non-polymorphic alleles (*nPOL*).

Fig. S3 The location of our observed genetic indices plotted in the space of indices calculated for 200 randomly selected replicates of our founding simulation (plotting all replicate runs is computationally and visually cumbersome).

Table S1 The number of alleles, effective number of alleles (N_e), allelic richness (Diversity), and the information index (I) observed per locus across all three sampled regions.

Table S2 Tests of departure from Hardy-Weinberg equilibrium across populations and loci.

Table S3 F_{ST}, standardized F_{STc}, and D_{est}, values across all three sampled regions.

Table S4 Parameters and parameter values of the simulation model that result in a total of 540 parameter combinations.

Table S5 The empirical genetic indices calculated from island individuals compared to the range of simulated values in the founding simulation (see above for explanation of variables).