

# Population history of the Hispaniolan hutia *Plagiodontia aedium* (Rodentia: Capromyidae): testing the model of ancient differentiation on a geotectonically complex Caribbean island

SELINA BRACE,\* IAN BARNES,\* ADAM POWELL,†‡ REBECCA PEARSON,§  
LANCE G. WOOLAVER,¶ MARK G. THOMAS,†\*\* and SAMUEL T. TURVEY††

\*School of Biological Sciences, Royal Holloway University of London, Egham TW20 0EX, UK, †Research Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK, ‡UCL Genetics Institute (UGI), University College London, Gower Street, London WC1E 6BT, UK, §Department of Respiratory Medicine, National Heart and Lung Institute, Imperial College London, St Mary's Campus, London W2 1PG, UK, ¶Wildlife Preservation Canada, 5420 Highway 6 North, Guelph, ON, Canada N1H 6J2, \*\*Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden, ††Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK

## Abstract

Hispaniola is a geotectonically complex island consisting of two palaeo-islands that docked c. 10 Ma, with a further geological boundary subdividing the southern palaeo-island into eastern and western regions. All three regions have been isolated by marine barriers during the late Cenozoic and possess biogeographically distinct terrestrial biotas. However, there is currently little evidence to indicate whether Hispaniolan mammals show distributional patterns reflecting this geotectonic history, as the island's endemic land mammal fauna is now almost entirely extinct. We obtained samples of Hispaniolan hutia (*Plagiodontia aedium*), one of the two surviving Hispaniolan land mammal species, through fieldwork and historical museum collections from seven localities distributed across all three of the island's biogeographic regions. Phylogenetic analysis using mitochondrial DNA (cytochrome *b*) reveals a pattern of historical allopatric lineage divergence in this species, with the spatial distribution of three distinct hutia lineages biogeographically consistent with the island's geotectonic history. Coalescent modelling, approximate Bayesian computation and approximate Bayes factor analyses support our phylogenetic inferences, indicating near-complete genetic isolation of these biogeographically separate populations and differing estimates of their effective population sizes. Spatial congruence of hutia lineage divergence is not however matched by temporal congruence with divergences in other Hispaniolan taxa or major events in Hispaniola's geotectonic history; divergence between northern and southern hutia lineages dates to c. 0.6 Ma, significantly later than the unification of the palaeo-islands. The three allopatric *Plagiodontia* populations should all be treated as distinct management units for conservation, with particular attention required for the northern population (low haplotype diversity) and the south-western population (high haplotype diversity but highly threatened).

*Keywords:* allopatric differentiation, ancient DNA, conservation genetics, Hispaniolan hutia, historical biogeography, palaeo-islands

Received 19 April 2011; revision received 18 January 2012; accepted 20 January 2012

Correspondence: Ian Barnes, Fax: +44 1784 414224;  
E-mail: ian.barnes@rhul.ac.uk

## Introduction

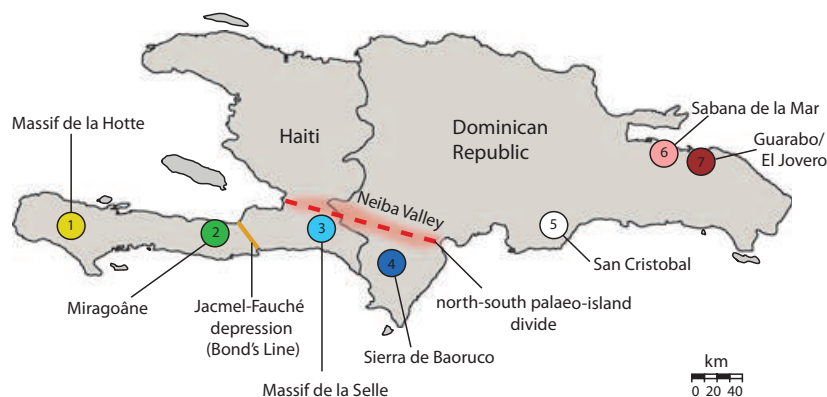
Nonvolant land mammals have rarely colonized non-continental islands. One of the few diverse radiations of endemic land mammals in this type of ecosystem took place in the Caribbean, which consists of a complex series of geotectonic units associated with an oceanic plate rather than the continental shelf regions of North or South America (Iturralde-Vinent & MacPhee 1999; Graham 2003). The Holocene insular Caribbean nonvolant land mammal fauna formerly consisted of ~120 endemic species of sloths, eulipotyphlan insectivores, primates and rodents (MacPhee 2009; Turvey 2009). However, over 100 mammal species or distinct island populations have become extinct following mid-Holocene Amerindian colonization and subsequent historical-era European settlement (MacPhee & Flemming 1999; MacPhee 2009; Turvey 2009), severely reducing the opportunity to investigate genetic patterns of mammalian evolution in the Caribbean region.

Our understanding of diversification and speciation in the recently extinct Caribbean land mammal fauna has been restricted by limited preservation of Tertiary fossils, uncertainty over Quaternary species diversity, and rapid degradation of ancient DNA in subtropical environments (Turvey 2009). In particular, it is unclear whether intransland speciation has been a significant factor in generating species diversity in the Caribbean mammal fauna. Several closely related species groups (e.g. capromyine, isolobodontine and plagiodontine hutias; heteropsomyine spiny rats; nesophontid island-shrews) apparently existed sympatrically in the Greater Antilles during the Late Quaternary, but species delimitations and phylogenetic interrelationships in these radiations are still unclear (Díaz-Franco 2001;

Condis Fernández *et al.* 2005). Although phylogeographic investigation of surviving Caribbean mammals has the potential to assess whether sister pairs of insular land mammal lineages arose *in situ* (e.g. through niche differentiation in response to topographic or ecological complexity) or through periods of interisland or intransland allopatry, studies addressing this significant evolutionary question have not yet been attempted.

Hispaniola, divided politically into the Dominican Republic and Haiti, is a large Caribbean island containing multiple endemic species pairs of many vertebrate taxa. Phylogenetic studies of nonmammalian endemic radiations have been conducted for several terrestrial vertebrate groups (e.g. birds: Townsend *et al.* 2007; Sly *et al.* 2010, 2011; reptiles: Glor *et al.* 2003; Gifford *et al.* 2004; Gifford & Larson 2008), to investigate biogeographic patterns of current-day species diversity across Hispaniola. It is also one of the few Caribbean islands to retain native land mammals: the Hispaniolan solenodon (*Solenodon paradoxus*), a basal eulipotyphlan insectivore, and the Hispaniolan hutia (*Plagiodontia aedium*), a capromyid rodent. Hispaniola therefore provides a rare opportunity within a noncontinental island system to test whether patterns and drivers of intransland mammalian diversification are congruent with other vertebrate groups, and whether this diversity evolved within continuously connected landmasses or in response to historical allopatric barriers to gene flow.

Hispaniola is topographically and ecologically heterogeneous and has a complex geotectonic history (Fig. 1). The island consists of independent northern and southern palaeo-islands that had docked by the late Miocene c. 10 Ma (Mann *et al.* 1991; Iturralde-Vinent & MacPhee 1999), but have subsequently remained separated by a



**Fig. 1** Map of Hispaniola, showing the collection localities for the *Plagiodontia* samples used in this study. Source localities of *Plagiodontia* specimens are indicated by numbered coloured circles: (yellow -1) Massif de la Hotte, (green -2) Miragoâne, (light blue -3) Massif de la Selle, (dark blue -4) Sierra de Baoruco, (white -5) San Cristobal, (pink -6) Sabana de la Mar, (maroon -7) Guarabo/El Jovero. The location of the Jacmel-Fauché depression, also known as Bond's Line, is indicated with a solid yellow line. The location of the Neiba Valley is illustrated in red with the bisecting broken red line indicating the position of the north-south palaeo-island divide.

prominent depression, the Neiba Valley or Cul-de-Sac/Enriquillo Graben (Fig. 1). This was at least periodically (if not permanently) inundated to form a narrow seaway until the late Pleistocene, providing a potential barrier to gene flow (Maurrasse *et al.* 1982; Graham 2003). The southern palaeo-island, a 350-km-long oceanic plateau terrane, experienced progressive uplift during the Neogene and formed a largely emergent peninsula by the Pliocene. This palaeo-island is subdivided into two major physiographic provinces, the Massif de la Hotte in the west and the Massif de la Selle and Sierra de Baoruco in the east (Fig. 1). These provinces are separated by the Jacmel-Fauché depression, a structural low bisecting the peninsula which was also inundated by a sea channel during some or all of the Plio-Pleistocene (Maurrasse *et al.* 1982).

The northern palaeo-island and the eastern and western regions of the southern palaeo-island are all biogeographically distinct, and these three regions are each characterized by substantial levels of endemism (e.g. Williams 1961; Schwartz 1980; Hedges 1999). Distinct northern and southern Hispaniolan biotas have been recognized since Mertens (1939), and the Jacmel-Fauché depression is sometimes referred to as a biogeographic boundary called 'Bond's Line' after the ornithologist James Bond (Latta *et al.* 2006). Recent genetic analysis of several Hispaniolan terrestrial birds and reptiles supports interpretation of these congruent patterns of intransland endemism as reflecting historical allopatry either across the north-south palaeo-island divide (Gifford *et al.* 2004; Townsend *et al.* 2007; Gifford & Larson 2008; Sly *et al.* 2011) or across Bond's Line (Sly *et al.* 2010, 2011). Similar *in situ* spatial processes of historical terrane accretion and marine inundation have been associated with allopatric speciation responsible for high levels of beta diversity across other complex islands such as New Guinea and Cuba (e.g. Heads 2001, 2002; Glor *et al.* 2004). However, some Hispaniolan birds show no evidence of lineage divergence associated with historical allopatry (Sly *et al.* 2011), making it difficult to predict whether similar patterns would be expected in the island's land mammal fauna.

Although two genera and species of extinct mammals (the capromyid rodent *Rhizoplagiodontia lemkei* and the primate *Insulacebus toussaintiana*) may have been biogeographically restricted to the Massif de la Hotte (Woods 1989; Cooke *et al.* 2011), very little is otherwise known about intransland distributions of most Hispaniolan mammals. Recovery of genetic material from Caribbean Quaternary fossils remains a major challenge, and so new studies of Hispaniola's cryptic and highly threatened extant land mammals (IUCN 2011) are required to understand comparative patterns of mammalian intransland evolution and biogeography. Ottenwalder

(2001) classified Hispaniolan solenodon populations from the northern and southern palaeo-islands as distinct subspecies on the basis of morphometric data, and morphological variation has also been reported in the Hispaniolan hutia. This threatened rodent species is distributed across all three of Hispaniola's biogeographic regions but has not been the subject of any recent studies, so that the relationship between the evolutionary pattern of hutia lineage divergence and Hispaniola's geotectonic history is still unknown.

The taxonomic history of the Hispaniolan hutia is confusingly complex, and multiple hypotheses have been proposed to describe apparent patterns of morphological variation across the island. *Plagiodontia aedium* was described from a specimen of unknown locality within Hispaniola and was subsequently associated with specimens from the north palaeo-island (Miller 1916). A new hutia population from the northern Dominican Republic was subsequently described as a separate species, *P. hylaeum* (Miller 1928). This led to a first biogeographic hypothesis that two *Plagiodontia* species occurred sympatrically across Hispaniola. Further discovery of living hutias in south-western Haiti apparently similar to the holotype of *P. aedium* led to the second biogeographic hypothesis that distinct species occurred allopatrically in different regions of Hispaniola (Johnson 1948). Anderson (1965) interpreted all living *Plagiodontia* populations as conspecific, but maintained the allopatric model by recognizing distinct southern Haitian (*aedium*) and Dominican (*hylaeum*) subspecies. Woods & Howland (1979) proposed the third biogeographic hypothesis that all living Hispaniolan hutias are conspecific with no recognizable subspecies. Confusion over the identity and relationship of living hutia populations has led some authors to refer to living Hispaniolan hutias simply as '*Plagiodontia* sp.' (Salazar 1977).

Here, we clarify the systematics and phylogeographic structuring of hutias across Hispaniola and investigate the congruence of evolutionary patterns within the Hispaniolan fauna, through genetic analysis of *Plagiodontia* individuals from both Haiti and the Dominican Republic. In particular, we aim to test between the three competing biogeographic hypotheses of spatial diversity patterns within *Plagiodontia*, by investigating (i) whether there is any evidence of major lineage divergence between populations across Hispaniola, and if so, (ii) whether such divergence is spatially congruent with Hispaniola's major geological boundaries and so can be interpreted as representing historical allopatry. Identifying the presence of evolutionarily distinct hutia populations across Hispaniola is also a pressing need before targeted conservation actions can be effectively implemented. However, given the difficulty of obtaining

extensive modern samples of this rare and cryptic mammal, such research is forced to utilize historical samples of varying quality in tandem with more recent material.

## Materials and methods

### Samples

We obtained a total of 20 hair, blood, soft tissue, bone and faecal samples from live-caught and dead hutias encountered during fieldwork in the Massif de la Hotte (Haiti) and Sierra de Baoruco (Dominican Republic) from 2005 to 2007 (Turvey *et al.* 2008). An additional 21 samples of soft tissue, tooth and bone from five further localities were also obtained from historical museum collections (Table 1), representing other regions across Hispaniola from which living hutia populations have been recently recorded (Sullivan 1983; Woods & Ottenwalder 1992). These 41 samples represent seven collection localities distributed across both palaeo-islands and both east and west of Bond's Line (Fig. 1). Wider geographic coverage would be desirable, but because of restricted availability of samples and the highly threatened status of the species, our samples represent the maximum coverage currently available.

### DNA extraction

Because of variation in sample preservation and quality, all DNA extractions were conducted in a dedicated ancient DNA laboratory (Royal Holloway, University of London), physically separated from the post-PCR laboratory. Bone samples were ground using a freezer mill (6750 SPEX CertiPrep) and liquid nitrogen. DNA extraction was based on Yang *et al.* (1998), a protocol that utilizes proteinase K to digest bone powders and silica spin columns to purify DNA, modified with inclusion of 1 M urea in the extraction buffer. Tissue and other

nonbone samples were extracted using QIAamp DNA Micro kit and protocols. Mitochondrial DNA (mtDNA) was amplified using overlapping fragments spanning the entire cytochrome *b* (cyt *b*) region (1140 base pairs). Because of the rarity of the Hispaniolan hutia, the majority ( $n = 38$ ) of available samples were highly degraded field-collected or historical tissues. Amplification of DNA was therefore restricted to short fragments of mtDNA because of the high copy number per cell of this genome compared to nuclear DNA. Primer pairs were designed specifically for this study to amplify overlapping fragments of between 200 and 837 base pairs across the cyt *b* region (see Table S1 for details, Supporting information). Initial reactions attempted to amplify the two longest overlapping fragments (837 and 760 base pairs), but because of DNA degradation, multiple primer pairs amplifying shorter fragments were generally employed. The total number of primers used for an individual sample ranged from 2 to 6 pairs, dependent on extent of mtDNA degradation. PCRs were performed using a final concentration of 1× PCR buffer, 0.2 μM of each primer, 250 μM dNTPs, 2 mM MgSO<sub>4</sub>, 1 mg/mL BSA, 1 Unit Platinum® *Taq* DNA Polymerase High Fidelity, purified water, and 2 μL of DNA extract in a 25-μL mix. PCR conditions were 5 min at 95 °C, followed by 40 cycles (increased to 55 cycles with low-quality samples) of 1 min at 92 °C, 1 min at 48–54 °C (dependent on primer pair specifications), 1 min at 68 °C, and with a final extension of 5 min at 68 °C.

Amplicons were purified using Exonuclease I and Shrimp Alkaline Phosphatase. Sequencing reactions were performed by Macrogen (Korea) using a high-throughput genetic analysis sequencer (ABI3730XL). Sequencing chromatograms were assembled and analysed using SEQUENCHER 4.0 analysis software (Gene Codes Corporation). Throughout our procedures, protocols to prevent contamination and ensure accurately coded (undamaged) mtDNA were followed: isolation of work

**Table 1** Details of the *Plagiodontia* samples used in this study; sample source (museum or field collected) and sample localities within Hispaniola.

Source	Country	Locality	SN	Palaeo-island	Bond's Line	<i>N</i>
FLMNH	Dominican Republic	San Cristobal	5	North	—	1
FLMNH	Dominican Republic	Sabana de la Mar	6	North	—	2
MCZ	Dominican Republic	Sabana de la Mar	6	North	—	5
USNM	Dominican Republic	Guarabo/El Jovero	7	North	—	10
USNM	Haiti	Miragoâne	2	South	West	1
FC	Haiti	Massif de la Hotte	1	South	West	17
FC	Dominican Republic	Sierra de Baoruco	4	South	East	3
FLMNH	Haiti	Massif de la Selle	3	South	East	2

FLMNH, Florida Museum of Natural History; MCZ, Museum of Comparative Zoology, University of Harvard; USNM, United States National Museum; FC, recent field collection; SN, site number (Fig. 1); *N*, number of samples.

areas, negative controls, reduced fragment length amplification and repeated PCR amplification and sequencing of fragments.

#### Population genetic analyses

Sequence diversity summary statistics were generated, with *Plagiodontia* samples allocated to one of either two or three allopatric groups (hereafter defined as 'populations') based on their geographic location. Two sets of allocations were investigated: the first dividing samples between northern and southern palaeo-islands, and the second further subdividing the southern population east and west of Bond's Line. Summary statistics calculated included number of haplotypes and nucleotide and haplotype diversity indices. As a means to test between competing hypotheses regarding the number of extant *Plagiodontia* species on Hispaniola, we compared our genetic divergence data with levels of inter- and intraspecific divergence shown by extant Cuban capromyids (Woods *et al.* 2001; Borroto-Páez *et al.* 2005), through pairwise estimates of average population sequence divergence in *cyt b* as calculated using the Kimura-2 Parameter (K2P)-corrected model (Kimura 1980) in ARLEQUIN Ver. 3.11 (Excoffier *et al.* 2005).

#### Phylogenetic analyses

*Plagiodontia* sequences were manually aligned with a sequence from the closely related Cuban hutia (*Capromys pilorides*) from GenBank (accession no. AF422915). Phylogenetic relationships were estimated using maximum likelihood (ML) and Bayesian methods. The DNA substitution model selected with MODELTEST 3.7 (Posada & Crandall 1998) under hierarchical likelihood ratio tests (hLRTs) was Tamura-Nei (TrN) with gamma distribution (shape parameter 0.0078). Maximum likelihood trees were generated in PAUP\* (Swofford 2000) using a full heuristic search with branch swapping by tree-bisection-reconnection (TBR). Bayesian trees were constructed using MrBayes 3.1 (Ronquist & Huelsenbeck 2003). Because MrBayes cannot implement the TrN+G model, we employed a similar nucleotide substitution model, general time reversible with gamma distribution (GTR+G), using four chains (three heated, one cold) that were run for  $1 \times 10^6$  generations, sampling every  $1 \times 10^3$  generation with a burn-in period of 250 trees. Nodal support was determined using ML bootstrap analysis with  $1 \times 10^3$  replicates in PAUP\* and by approximate posterior probabilities performed in MrBayes. *Capromys pilorides* was used as the outgroup for both sets of analyses. Connection lengths and alternative links for a minimum-spanning network of haplotypes were generated in ARLEQUIN to visualize sequence diversity.

Estimating the timing of lineage divergence is problematic because of the absence of a Tertiary fossil record for Hispaniolan land mammals (and an extremely limited Tertiary record for any capromyids; MacPhee & Iturralde-Vinent 1995) with which to calibrate a molecular clock. To provide a divergence date estimate, our analyses are therefore restricted to the use of a fixed mutation rate. The choice of mutation rate can also be controversial, as rodents exhibit a wide range of substitution rates per third codon position per million years (average = 0.176 substitution/site/Myr) (Nabholz *et al.* 2008). The molecular clock in *Plagiodontia* is likely to run relatively slowly, as hystricognath rodents have unusually low metabolic rates, long gestation times and are relatively long-lived (Woods *et al.* 2001). We therefore apply a rate of 4% per site per million years, representing a moderate rodent mutation rate for *cyt b* (Irwin *et al.* 1991; Hadly *et al.* 2004). While implementation of a relaxed clock would be desirable to test validity of the applied mutation rate, this is also prevented by the absence of any suitable fossil calibration. In an attempt to address this issue, we apply two analyses. The first estimates the timing of lineage divergence based on a fixed mutation rate. The second estimates the mutation rate required to make the divergence date between northern and southern *Plagiodontia* lineages temporally congruent with the docking of the northern and southern palaeo-islands (10 Ma). All divergence estimates were calculated in BEAST (Drummond & Rambaut 2007) under a GTR+G substitution model (Lanave *et al.* 1984), using a strict molecular clock and either a 4% mutation rate or a fixed divergence date. Chain length was set to  $1 \times 10^8$  generations, with data collected every  $1 \times 10^3$  generations and a burn-in of  $1 \times 10^5$  generations. Outputs from both the MrBayes and BEAST MCMC runs were examined with Tracer v1.5 (Rambaut & Drummond 2007) to evaluate convergence and effective sample sizes.

#### Coalescent modelling

To test the robustness of our phylogeographic inferences and gain the maximum signal from our data, we applied a coalescent-based (Wakeley 2009) modelling approach to our data set using two plausible models of *Plagiodontia* demographic history. Data sets for each model were simulated in Serial SimCoal (SSC; Anderson *et al.* 2005), a coalescent-based programme, with parameters estimated using approximate Bayesian computation (ABC; Beaumont *et al.* 2002). Our models were then compared using an approximate Bayes factor method (see Ray *et al.* 2010), and summary statistics of the observed data were compared with those of the model data sets (with their associated parameter value

estimates) using the method proposed by Voight *et al.* (2005).

The first model (model 1) was designed to test whether the most basic pattern of allopatric differentiation could be inferred from our data: lineage divergence between two populations across the major geological boundary of Hispaniola (Neiba Valley, the north–south palaeo-island divide) (Fig. 1). This two-population model (representing northern and southern palaeo-islands) assumed divergence from a common ancestral population 1 666 667 generations ago, equivalent to 10 Ma (reflecting the approximate timing of docking of the palaeo-islands and thus the earliest possible timing for colonization to have occurred), and assuming a generation time for *Plagiodontia* of 6 years (Kleiman *et al.* 1979; Nowak 1999). Parameter values (migration rate and  $N_e$ ) were estimated using ABC with uniform prior distributions for each; these priors were chosen as they assume the least previous knowledge. The two populations were assumed to have constant (the simplest model and a reasonable assumption for a small mammal species, the population size of which would swiftly reach equilibrium on an island) but unknown effective female population sizes ( $N_e$ ; values between 1 and  $5 \times 10^5$  were explored independently for each). Bidirectional background migration was assumed, because the two populations diverged from a common ancestral population. Values of  $0\text{--}5 \times 10^{-5}$  per generation were selected for exploration after initial tests with wider ranges produced approximate posterior density rates well below the upper bounds found in the *Plagiodontia* data set.

The second model (model 2) was designed to test a more complex pattern of allopatric differentiation incorporating an additional geological division (Bond's Line; Fig. 1) previously associated with population divergence (Sly *et al.* 2010, 2011). This model was therefore the same as the first except it assumed the divergence of three rather than two populations (representing the northern palaeo-island and the eastern and western regions of the southern palaeo-island). The population west of Bond's Line was assumed to have diverged from the population east of Bond's Line 1 666 661 generations ago, six generations (36 years) after unification of the two palaeo-islands (our estimate of the earliest possible migration date), which in turn was assumed to have diverged from the northern population 1 666 667 generations ago. All three populations were again assumed to be of constant but unknown  $N_e$  (values between 1 and  $5 \times 10^5$  were again explored independently for each). Bidirectional background migration was assumed between populations east and west of Bond's Line (values between 0 and  $5 \times 10^{-4}$  per generation were explored), and between populations east of Bond's Line and on the northern palaeo-island (values

between 0 and  $5 \times 10^{-5}$  per generation were explored). For both models, a mutation rate of 4% per million years, a transition:transversion rate of 17.1:1 (ARLEQUIN) and rates to be gamma distributed with parameter 0.094 and 4 rate classes were assumed. For both models,  $4.5 \times 10^6$  coalescent simulations were performed.

Sequences were sampled from coalescent simulations according to the number of sequences generated in this study. Two samples were taken from the southern palaeo-island simulated population ( $n = 5$  and 11) and one from the northern palaeo-island simulated population ( $n = 11$ ). Within-sample summary statistics (number of haplotypes, number of segregating sites, average pairwise difference, haplotype diversity (biased by  $n-1/n$ ), Tajima's  $D$ ) and between-sample summary statistics (number of haplotypes private to each sample, average pairwise difference,  $F_{ST}$ ) were calculated from the observed data using ARLEQUIN and according to the Bayesian SSC formulae (Excoffier *et al.* 2000; Anderson *et al.* 2005). Values for all ABC target statistics from the empirical data are reported in the supplemental material (Tables S2 and S3, Supporting information). Our model parameters were estimated by ABC (Beaumont *et al.* 2002), conditioning on the same summary statistics as listed above for the observed data. We note that while the Tajima's  $D$  statistic (Tajima 1989) is a derivative of  $S$  and  $\pi$ , the ABC algorithm results in additional conditioning information from the inclusion of the Tajima's  $D$  statistic.

Marginal posterior distributions were then estimated for the parameters of each model. The approximate joint posterior distribution was first obtained by performing a local-linear multivariate weighted regression adjustment (Beaumont *et al.* 2002), from which the marginal posterior distributions were derived for each parameter.

Comparisons between the two models were made by calculating the Euclidean distance (Ray *et al.* 2010) between observed and simulated summary statistics for each of the total  $9 \times 10^6$  simulated data sets. With tolerance proportion  $F_\delta = 0.001$ , the  $4.5 \times 10^3$  best-fitting parameter sets from each of the two models were retained and placed into a common pool. These  $9 \times 10^3$  simulations were then ordered by increasing Euclidean distance, which was recalculated after normalization of summary statistics using means and standard deviations recomputed on the pool. Relative proportion of simulations from each model within the  $n$ -smallest Euclidean distances gave an estimate of the posterior probability of each model (Estoup *et al.* 2004; Ray *et al.* 2010).

In the above analysis, two-population and three-population models were assumed to be the simplest models of the biogeographic structuring of *Plagiodontia* populations over the last 10 Myr. Nonetheless, it is

possible that misspecified models have been used, which would lead to misleading inferences on the population history of *Plagiodontia*. To examine this possibility, Fisher's method was applied to combine two-tailed probabilities of the observed conditioning statistics, obtained by comparison to simulation using modes of the parameter estimates obtained by ABC for the best model. The resultant  $\chi^2$  values were compared to those obtained by comparing each simulation against the set of all other simulations for each model (Voight *et al.* 2005) to obtain a two-tailed probability of the statistics of the observed data under the best model and best estimates of the model parameter values.

## Results

### DNA sequence data

From the 41 *Plagiodontia* individuals sampled, 27 successfully yielded mtDNA for the entire *cyt b* gene (see Table S4 for details, Supporting information). Only two of these 27 individuals, both recently field-collected samples, had sufficient mtDNA preservation to permit amplification of the two longest mtDNA fragments (837 and 760 base pairs) that comprised the entire *cyt b* region. The remaining eleven field-collected and 14 museum specimens used in these analyses required amplification using additional (between 4 and 6) primer pairs to recover the same region of mtDNA. These 27 successful samples represent six of the seven sampling localities under investigation; the single available sample from San Cristobal (Fig. 1) did not yield amplifiable mtDNA. A total of 18 haplotypes were identified (Genbank accession nos JQ410001–JQ410018: see Table S4, Supporting information). Haplotype diversity indices were high (0.70–0.95) for all regions analysed. However, nucleotide diversity was markedly lower in the northern population (0.001) compared with the southern populations (south-eastern = 0.016, south-western = 0.013; Table 2). Pairwise estimates of sequence divergence ranged from 1.03% (between south-eastern and south-western populations) and 3.2% (between south-western and northern populations; Table 3).

### Phylogenetic analyses

Maximum likelihood and Bayesian analyses generated congruent phylogenetic trees (Fig. 2). Inclusion of a minimum-spanning network (Fig. 3) provides further comparative representation of genetic differences between allopatric *Plagiodontia* populations, with southern populations exhibiting greater interhaplotype diversity (1–23 mutational steps between neighbouring haplotypes) than the northern population (one muta-

**Table 2** Summary statistics of the molecular diversity (*cyt b*) within regionally sampled *Plagiodontia* from Hispaniola.

Region	<i>N</i>	<i>H</i>	<i>h</i>	$\pi$
Northern palaeo-island	11	7	0.8182 (0.1191)	0.001085 (0.000837)
Southern palaeo-island (east of Bond's Line)	5	3	0.7000 (0.2184)	0.016491 (0.010342)
Southern palaeo-island (west of Bond's Line)	11	8	0.9455 (0.0535)	0.012855 (0.007041)
Southern palaeo-island (entire)	16	11	0.9500 (0.0364)	0.018604 (0.009710)

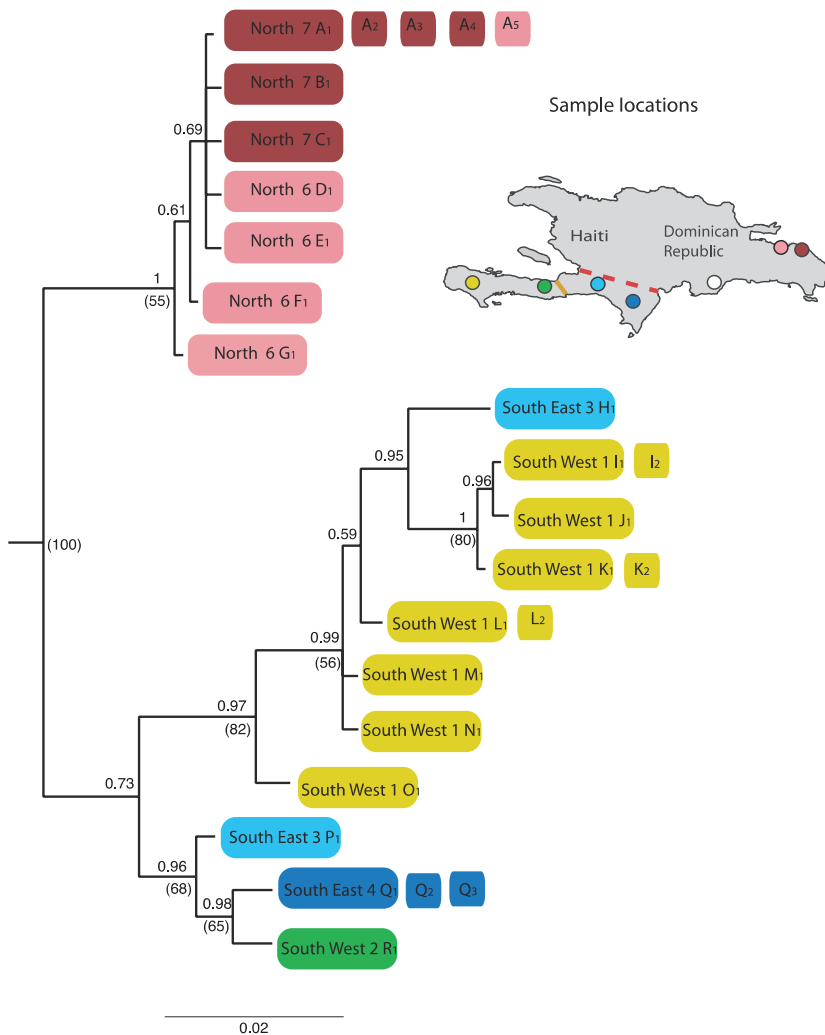
Molecular diversity indices (*N*, individuals; *H*, haplotypes; *h*, haplotype diversity;  $\pi$ , nucleotide diversity; parentheses, standard error).

tional step between neighbouring haplotypes). All phylogenetic analyses recovered the same clades. We report strong Bayesian approximated posterior probability values for the monophyly of the northern clade (1). The monophyly of the southern clade is less well supported (0.73); however, two distinct southern lineages (east and west) are well supported (0.97 and 0.96). Greater nodal support values are reported from the Bayesian analysis compared with the bootstrap analysis, although this is not an unexpected result as the conservative nature of bootstrapping is a well-reported phenomenon (Erixon *et al.* 2003). The northern clade contains seven haplotypes (11 samples) from two localities, and the southern clade contains 11 haplotypes (16 samples) from four localities (two localities on either side of Bond's Line). The southern clade consists of two distinct subclades that are closely but not completely congruent with the east-west biogeographic division across Bond's Line. The two samples that did not conform to this trend (South West 2 and South East 3), which were nested within subclades otherwise comprising samples from the other side of Bond's Line, were collected from Miragoâne and the Massif de la Selle, the two sites that are geographically closest to Bond's Line.

Divergence date estimates indicate that *Plagiodontia* lineage separation between northern and southern palaeo-island populations occurred during the Pleistocene between 0.433 and 0.765 Ma (95% HPD), with a mean estimated value of 0.594 Ma. East-west lineage separation in the southern palaeo-island occurred between 0.312 and 0.572 Ma (95% HPD), with a mean estimated value of 0.436 Ma. The most recent common ancestor of the sampled northern palaeo-island individuals dates from between 0.022 and 0.088 Ma (95% HPD), with a

**Table 3** Average pairwise estimates of sequence divergence (cyt *b*) between regionally sampled *Plagiodontia* from Hispaniola.

	Northern palaeo-island	Southern palaeo-island (east of Bond's Line)	Southern palaeo-island (west of Bond's Line)	Southern palaeo-island (entire)
Northern palaeo-island		2.87%	3.20%	2.86%
Southern palaeo-island (east of Bond's Line)			1.03%	

**Fig. 2** Phylogeny of *Plagiodontia* mitochondrial haplotypes (cyt *b*). Numbers above the nodes represent Bayesian posterior probabilities; numbers in parentheses represent bootstrap values (only values above 50% are shown). The out-group *Capromys pilorides* is removed for display purposes. Each haplotype is identified through a unique letter (A-R; see Table S4 for details, Supporting information) additional nomenclature, and the colour corresponds to the geographical origin of the sample (Fig. 1 and inset map). Adjacent coloured blocks indicate the number of additional samples with the same haplotype A-R, individually identified through a unique subscript number, with the colour of each block corresponding to the geographical origin of the sample.

mean estimated value of 0.052 Ma (Table 4). Analysis using a fixed date (10 Ma) for lineage divergence between northern and southern palaeo-island populations required a mean mutation rate of 0.22% per site per million years (95% HPD 0.168–0.284).

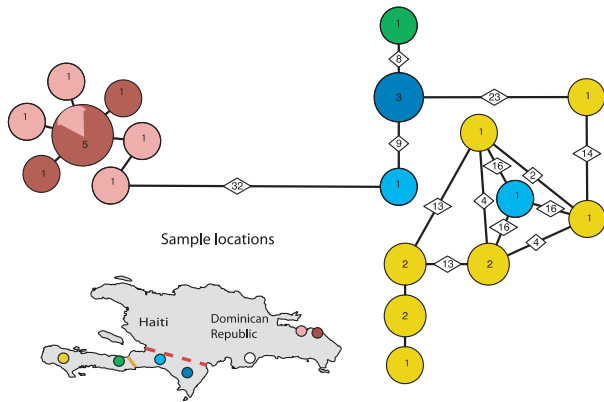
#### Coalescent-based analyses

Models 1 and 2 both estimated that  $N_e$  for the northern palaeo-island was lower than for the southern palaeo-island (model 1) or for the combined southern palaeo-

islands (model 2). Both models also estimated low migration rates across both biogeographic boundaries, suggesting limited contact between populations (Figs 4 and 5; Table 5).

Results from Bayes factor analysis (Fig. 6) showed greater support, through higher marginal posterior probability values, for the three-population model (model 2) compared with the two-population model (model 1). In this model, the modal  $N_e$  (and associated 95% credibility intervals) were 7477 (3112–35,393), 6509 (1650–56,207) and 18,086 (5849–66,407) for northern,





**Fig. 3** Minimum-spanning network derived from *Plagiodontia* mitochondrial haplotypes (*cyt b*). The numbers within circles are the total number of samples represented by each haplotype. Each line within the network represents a single mutational change; when a line represents multiple mutational steps this is indicated by a diamond, the number within indicating the total number of mutational steps. Colours correspond to the geographical origin of the sample (Fig. 1 and inset map).

**Table 4** The estimated lineage divergence dates (*cyt b*) for regionally sampled *Plagiodontia* from Hispaniola.

	Divergence dates (Ma)		
	95% HPD lower	95% HPD upper	Mean
Northern and southern palaeo-islands	0.433	0.765	0.594
East and west of Bond's Line	0.312	0.572	0.436
Northern palaeo-island	0.022	0.088	0.052

south-eastern and south-western palaeo-islands, respectively. Estimated modal migration rates (and associated 95% credibility intervals) between northern and south-eastern, and between south-eastern and south-western palaeo-island populations were  $9 \times 10^{-6}$  ( $1.7 \times 10^{-6}$  to

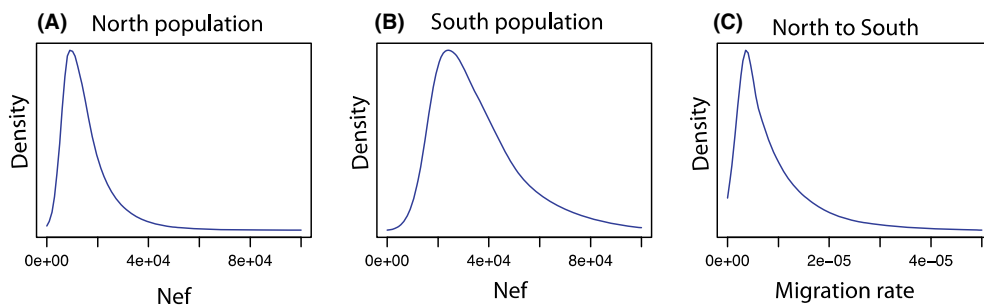
$3.37 \times 10^{-5}$ ) and  $4.57 \times 10^{-5}$  ( $1.11 \times 10^{-5}$  to  $2.78 \times 10^{-4}$ ) per generation, respectively. The three-population model with parameter values taken as modes of the posterior estimates was not rejected by the method of Voight *et al.* (2005), returning a two-tailed p-value of 0.9173.

**Discussion**

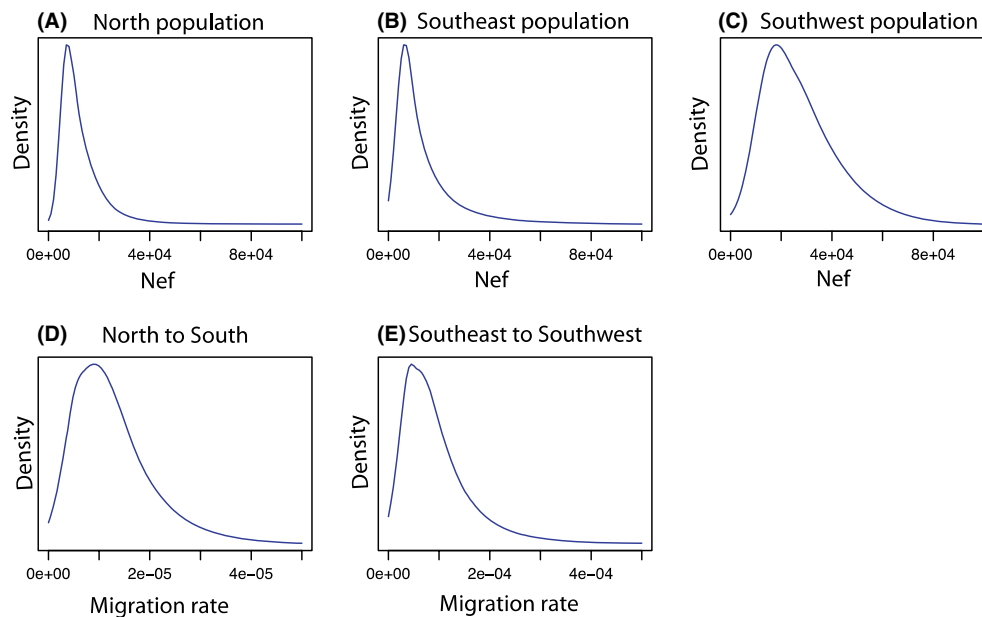
*Historical biogeography*

Our results demonstrate a high level of phylogeographic structure within *Plagiodontia*, with a spatial pattern of lineage divergence that is biogeographically consistent with the geotectonic history of Hispaniola. These results therefore support the second phylogenetic hypothesis originally proposed for *Plagiodontia*, that distinct hutia sister taxa occur allopatrically in different regions of the island (Johnson 1948; Anderson 1965). The primary phylogenetic division within the genus is between individuals from the northern and southern palaeo-islands (Figs 2 and 3). A further, near-complete subdivision of the southern population across Bond's Line (Fig. 1) into eastern and western lineages is also demonstrated.

Coalescent simulations across a range of migration values were used to investigate migration between *Plagiodontia* populations. However, only simulations with very low migration rates achieved a good fit to the observed data from the northern to the southern population (Fig. 4) and from northern to south-eastern and from south-eastern to south-western populations (Fig. 5), suggesting minimal migration between all populations under investigation. The ecology of *Plagiodontia* is poorly understood, but it is thought to exist in family-based social groups (Radden 1967; Woods 1981) similar to other hutia species (Clough 1972; Anderson *et al.* 1983; Silva Taboada *et al.* 2007), suggesting that it is not a solely male-dispersal strategist. We propose that *Plagiodontia* populations from the three biogeographic regions of Hispaniola are probably genetically isolated from each other. In the three-population model,



**Fig. 4** Posterior probability distributions of  $N_e$  and migration rate for model 1 (two populations). Posterior probability density curves are plotted against female effective population size in the northern palaeo-island (A) and the southern palaeo-island (B). Posterior probability density curves are plotted against migration rate from the northern to the southern palaeo-island (C).



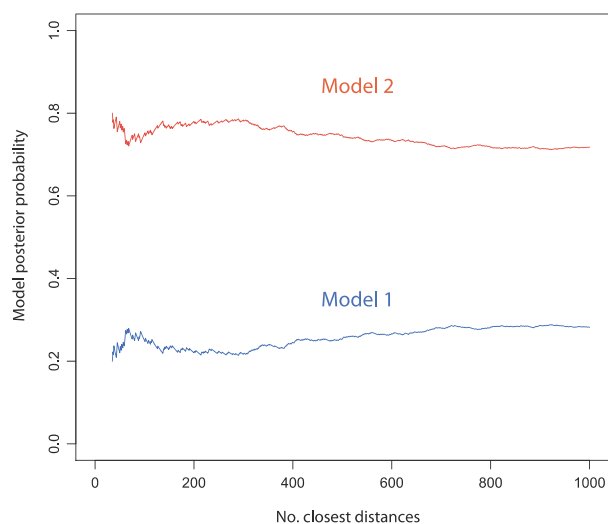
**Fig. 5** Posterior probability distributions of  $N_e$  and migration rate for model 2 (three populations). Posterior probability density curves are plotted against female effective population size in the northern population (A), the south-eastern population (B) and the south-western population (C). Posterior probability density curves are plotted against migration rates from northern to the south-eastern populations (D) and south-eastern to south-western populations (E).

Population	Parameter	Quantile		Mode
		2.50%	97.50%	
<b>Model 1</b>				
Northern	$N_e$	3888	40 848	9192
Southern	$N_e$	11 957	83 438	23 909
Northern to southern	Migration rate (per generation)	0.0000008	0.0000336	0.0000037
<b>Model 2</b>				
Northern	$N_e$	3112	35 393	7477
South-eastern	$N_e$	1650	56 207	6509
South-western	$N_e$	5849	66 407	18 086
Northern to south-eastern	Migration rate (per generation)	0.0000017	0.0000337	0.0000090
South-eastern to south-western	Migration rate (per generation)	0.0000111	0.0002778	0.0000457

**Table 5** Quantile and modal values of the posterior probability distribution for  $N_e$  and migration rate for regionally sampled *Plagiodontia* from Hispaniola.

estimated migration rates between northern and south-eastern populations were very low (modal rate =  $9 \times 10^{-6}$ ); whereas migration across Bond's Line was estimated to be almost an order of magnitude higher (modal rate =  $4.57 \times 10^{-5}$ ), this rate still constitutes an exceptionally low level of migration between populations. Population division across Bond's Line is further supported by comparative Bayes factor analyses (Fig. 6), where the three-population model (with more population substructure and two independent/distinct migration rates) has higher posterior probability support than the simpler two-population model. Although

the models examined here are, necessarily, oversimplifications of the true demographic history of *Plagiodontia* populations, the summary statistics of the data are a typical realization under model two with modal parameter estimates, so that this model cannot be rejected with our data. While our inferences have been made using models that assume neutrality, it is possible that positive natural selection has acted on mtDNA in *Plagiodontia* populations; a lack of recombination means that any positively selected variants would carry the rest of the mtDNA genome with them. However, we note that the most extreme Tajima's  $D$  value calculated from our



**Fig. 6** The approximate posterior probability for models 1 and 2 as dependent on the number of closest distances. The blue line indicates posterior probability values for the two-population model (model 1). The red line indicates posterior probability values for the three-population model (model 2).

data in any of the subpopulations is  $-1.56949$ , which is within the range expected for neutral regions ( $-2$  to  $2$ ) (Tajima 1989).

The pattern of lineage divergence observed in *Plagiodontia* between Hispaniola's northern and southern palaeo-islands is spatially congruent with that shown by several other vertebrate taxa and demonstrates the importance of historical allopatry in driving differentiation and lineage accumulation on large, geologically complex islands. However, this spatial congruence is not matched by temporal congruence in lineage divergence between *Plagiodontia* and most other Hispaniolan taxa for which phylogeographic studies have been conducted. Divergences between northern and southern populations of other Hispaniolan vertebrates have been dated to the Miocene (chat-tanager, *Calyptophilus*: 9.7 Ma; Townsend *et al.* 2007), Pliocene (teiid lizard, *Ameiva chrysolaela*: 4.3 Ma; Gifford *et al.* 2004), middle Pleistocene (narrow-billed tody, *Todus angustirostris*: 1.5 Ma; Sly *et al.* 2011) and late Pleistocene (green-tailed ground-warbler, *Micrologia*: 0.5 Ma; Sly *et al.* 2011). Estimated divergence between *Plagiodontia* lineages occurred at the latter end of this range, *c.* 0.6 Ma (Table 4); the mutation rate required for north/south lineage divergence to be fixed at 10 Ma is 0.22% per site per million years, an order of magnitude slower than moderate estimates for rodent mutation rate for *cyt b* (Irwin *et al.* 1991; Hadly *et al.* 2004), thus rejecting a temporally congruent divergence with Miocene docking of the palaeo-islands. This variation in phylogeographic divergence dates across Hispaniola's main geotectonic division suggests that the marine channel

between the northern and southern palaeo-islands, and subsequent inundation of the Neiba Valley throughout the Neogene and Quaternary, acted as a semi-permeable barrier to gene flow that permitted periodic colonization events to occur throughout its geological history either via overwater dispersal (cf. Hedges 2006) or intermittent regional marine regression. Similar patterns of asynchronous, progressive colonization across marine barriers have also been demonstrated for the faunal history of other island systems such as Madagascar (Poux *et al.* 2005; Ali & Huber 2010).

The more recent divergence between *Plagiodontia* populations across the north-south palaeo-island boundary may reflect ecological differences between this mammal and most other Hispaniolan taxa for which phylogeographic data are available. *Calyptophilus* and *Todus angustirostris* are high-elevation dense forest specialists with limited dispersal ability and disjunct modern-day distributions across Hispaniolan mountain ranges (Latta *et al.* 2006; Townsend *et al.* 2007), and *Ameiva chrysolaela* occurs only in xeric lowlands (Gifford & Larson 2008). In contrast, *Plagiodontia* may be an ecological generalist; although its current distribution and habitat preferences remain poorly understood, it has been recorded across a range of habitat types and elevations from mangrove swamp to montane forest (Miller 1928; Woods 1981; Sullivan 1983; Woods *et al.* 1985; Turvey *et al.* 2008). This wide habitat tolerance may have provided *Plagiodontia* with greater ecological flexibility for occupying and colonizing habitats on either side of the Neiba Valley throughout periods of environmental change across the Neogene and Quaternary, making Hispaniola's geotectonic and biogeographic boundaries less likely to act as significant barriers to gene flow over geological time. *Micrologia*, which also shows a late Pleistocene divergence across the Neiba Valley, has a similarly broad ecological distribution across different habitats (Sly *et al.* 2011). However, this hypothesis may be challenged by the different patterns of phylogenetic distinctiveness shown by southern palaeo-island populations of *Plagiodontia* and *Calyptophilus*; whereas *Plagiodontia* contains distinct eastern and western clades, *Calyptophilus* populations exhibit no genetic distinction across Bond's Line (Townsend *et al.* 2007).

#### Taxonomy and conservation

Our combined analyses support spatial lineage distinction across Hispaniola and thus some support for species-level distinction between *Plagiodontia* populations. However, our estimates of sequence divergence, 2.86% between northern and southern populations and 1.03% between southern populations (Table 3), would constitute the lowest interspecific divergence value currently

recorded for the Capromyidae (3.0–20.4%: Woods *et al.* 2001; Borroto-Páez *et al.* 2005). In the absence of genetic data from the nuclear genome and/or consistent morphometric variation differentiating *Plagiodontia* populations (Anderson 1965; Woods & Howland 1979), we therefore conservatively recognize a single living Hispaniolan hutia species containing two distinct subspecies, *P. aedium aedium* Cuvier, 1836 (southern lineages, following usage of previous authors) and *P. aedium hylaeum* Miller 1928 (northern lineage).

Recognition of distinct *Plagiodontia* lineages is highly important for conservation prioritization. *Plagiodontia aedium* represents one of Hispaniola's last two surviving endemic land mammals and is classified as Endangered (IUCN 2011). Despite our conservative taxonomic approach, we stress that the three allopatric *Plagiodontia* populations should all be treated as distinct management units in accord with their observed genetic differentiation and associated geographic separation. We also highlight striking disparities in both within-clade phylogenetic structure and estimated effective population size between different *Plagiodontia* populations. Estimates of  $N_e$  in the northern population are low (Table 5) with samples exhibiting low nucleotide diversity and haplotypes configured in a star-like topology (Figs 2 and 3), a pattern often associated with demographic decline followed by expansion (Avise 2000). Our temporal estimate of population divergence in the north (0.052 Ma) represents a period of major oscillation in sea-surface temperature in the subtropical North Atlantic (Sachs & Lehman 1999), and climatic fluctuation throughout the middle-late Pleistocene may have driven local population fragmentation and extirpation in many Caribbean taxa (Getty *et al.* 2001). The true diversity of the northern population may have been underestimated because of the relatively restricted region from which the available samples originated. However, samples from the south-western population are from a similarly restricted geographic area (the Duchity region of the Massif de la Hotte; Turvey *et al.* 2008), and our results indicate markedly greater diversity and higher  $N_e$  in this region (Figs 2 and 3, Table 5). The Massif de la Hotte is a globally important biodiversity hotspot containing extremely high levels of range-restricted endemic species (Ricketts *et al.* 2005). Efforts to maintain this genetically diverse hutia population with the wider ecosystems of the Massif de la Hotte, and to further survey the northern subspecies to establish its current distribution and abundance, should therefore represent urgent conservation priorities.

The identification of distinct conservation-relevant units in *Plagiodontia*, a rare and highly threatened mammal, was only feasible through utilization of a wide range of degraded archival material, including soft

tissue, hair, bone, teeth and faeces. While it would be desirable to confirm our conclusions through corroborative data from nuclear genes, the only currently available material is insufficiently preserved to enable reliable data to be obtained. However, with the application of recently developed analytical tools, including coalescent modelling and ABC, we have been able to maximize our use of the available mitochondrial data. These results support our phylogenetic inference of lineage separation congruent with ancient geographic boundaries on Hispaniola, further highlighting the level of population isolation and providing estimates of effective population sizes. This combination of techniques has enabled us to explore the population history of one of the last endemic land mammals of the insular Caribbean and to reveal the importance of allopatric divergence in Hispaniolan mammal evolution associated with the island's complex biogeographic history.

### Acknowledgements

Funding for this study was provided by the Centre for Ecology and Evolution, SYNTHESYS2 made available by the European Community Research Infrastructure under FP7 ('Synthesis of Systematic Resources', 226506-CP-CSA-Infra) and the Natural Environment Research Council (NERC Postdoctoral Research Fellowship IP/1075/1108 to STT; NERC Doctoral Training Grant NER/S/A/2006/14031 to SB). Logistical support for fieldwork in Haiti to collect hutia samples was provided by Société Audubon Haïti and Sociedad Ornitológica de la Hispaniola; particular thanks go to Helen Meredith, Paul Scofield, Jorge Brocca, Eladio Fernandez, Nicolas Corona, Kate Wallace, Osé Pauléus, Philippe Bayard, Jessie Haspil, Paul Judex Ezoardin, Potau Roseval, Frederique Chéron, José Ottenwalder and Carly Waterman. We thank Linda Gordon and Mike Carleton (USNM), Judy Chupasko and Hopi Hoekstra (MCZ) and Candace McCaffery and David Reed (FLMNH) for providing further hutia samples. We also thank Richard Young, John Fa and Meirav Meiri for technical assistance and project development.

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S.B. is a biologist, interested in the use of ancient and historical molecular data to infer evolutionary processes and species his-

ories. I.B. is an evolutionary biologist specialising in the use of ancient DNA to resolve population and species-level processes. A.P. is a population geneticist and gene-culture coevolutionist, interested in inferring the demographic history of species using both genetic and archaeological data. R.P. has a continuing interest in evolutionary biology and the use of molecular markers to infer phylogenetic histories and is currently working on cellular and molecular mechanisms of infection. L.W. is a conservation biologist experienced in reintroductions, adaptive management and community conservation of critically endangered species. M.T. is Professor of Evolutionary Genetics and is interested in evolutionary inference from ancient and modern genetic and cultural variation data. S.T.T. is a conservation biologist and palaeontologist with an interest in vertebrate evolution and extinction on island systems.

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### Data accessibility

Haplotype sequences have been deposited in Genbank with accession nos JQ410001–JQ410018.

### Supporting information

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

**Table S1** Details of primer pairs used to amplify mtDNA from Hispaniolan hutia *Plagiodontia* (cytochrome *b*).

**Table S2** Within population ABC target statistics for *Plagiodontia* regionally sampled from across Hispaniola.

**Table S3** Between populations ABC target statistics for *Plagiodontia* regionally sampled from across Hispaniola.

**Table S4** Details of *Plagiodontia* samples from Hispaniola that successfully yielded mitochondrial DNA (cytochrome *b*).

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