High gene flow across heterogeneous tropical montane environments in a Bornean endemic small mammal

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Abstract

Rapid shifts in environmental variables associated with elevational changes in montane ecosystems provide opportunities to test hypotheses regarding the effects of environmental heterogeneity on gene flow and genetic structure. In tropical mountains, spatial environmental heterogeneity combined with seasonal environmental stability is predicted to result in low dispersal across elevations. Few studies have investigated the genetic consequences of elevational environmental heterogeneity in tropical montane mammals. Here, we use a population genomics approach to test the hypothesis that mountain treeshrews (*Tupaia montana*) exhibit limited gene flow across elevational gradients and between two neighboring peaks within Kinabalu National Park (KNP) in Borneo. We sampled 83 individuals across elevations on Mt. Tambuyukon (MT) and Mt. Kinabalu (MK) and sequenced mitogenomes and 4,106 ultraconserved elements containing an average of 1.9 single nucleotide polymorphisms per locus. We detected high gene flow across elevations and between peaks. We found greater genetic differentiation on MT than MK despite its lower elevation and associated environmental variation. This implies that, contrary to our hypothesis, genetic structure in this system is not primarily shaped by elevation. We propose that this pattern may instead be the result of colonization history combined with restricted upslope gene flow on MT due to unique plant communities associated with its upper montane habitats. Our results serve as a foundation to identify and mitigate future effects of climate change on mountain treeshrews in KNP. Given predictions for 2100 CE, we predict that mountain treeshrews will maintain genetic connectivity in KNP, making it an important conservation stronghold.

Introduction

Montane ecosystems are highly valuable to the study of evolutionary and genetic consequences of environmental heterogeneity due to the rapid shifts in environmental variables (e.g. temperature, precipitation, and solar radiation) over short distances. Relative to those in the temperate zone, tropical mountains experience less seasonal temperature variation which generates greater elevational stratification (Polato et al., 2018). Janzen (1967) hypothesized that this temporal thermal stability paired with spatial environmental heterogeneity should select for narrow thermal tolerances, which in turn result in low effective dispersal and population isolation across elevational gradients (Ghalambor, Huey, Martin, Tewksbury, & Wang, 2006; Gill et al., 2016). Consistent with Janzen's (1967) hypothesis, many studies have documented narrower elevational ranges among tropical montane species than temperate species across diverse taxonomic groups (McCain, 2009; Ghalambor et al., 2006). Fewer studies have tested the prediction stemming from Janzen's (1967) hypothesis that restricted gene flow among populations spanning elevational gradients results in genetic divergence. The available data regarding this prediction are contradictory - some studies have found

significant population genetic divergence across elevations, for example, in insects (Polato et al., 2018; Gueuning et al., 2017) and birds (Gadek et al., 2018; DuBay & Witt, 2014; Linck, Freeman, & Dumbacher, 2019). Others have found high rates of gene flow, sometimes in the presence of adaptive phenotypic divergence (Cheviron & Brumfield, 2009; Gadek et al., 2018; Branch, Jahner, Kozlovsky, Parchman, & Pravosudov, 2017).

Very few studies have investigated the population genetic structure of small mammals across elevational gradients in tropical montane ecosystems (Muenchow, Dieker, Kluge, Kessler & von Wehrden, 2018; but see Yu, 1995). We address this knowledge gap by elucidating the population genetic structure of the mountain treeshrew, *Tupaia montana* , across its full elevational range on two mountains in Kinabalu National Park (KNP), Sabah, Borneo: Mt. Kinabalu and Mt. Tambuyukon (Figure 1). The mountain treeshrew provides an interesting system in which to study the effect of environmental gradients on population structure because it has a broad elevational distribution compared to other small mammals in KNP (Camacho-Sanchez, Hawkins, Tuh Yit Yu, Maldonado, & Leonard, 2019; Nor, 2001).

Understanding the population genetic structure of tropical montane taxa like the mountain treeshrew is important for conservation because it enables researchers to identify metapopulation dynamics and distinct evolutionary units warranting protection and to predict and track species' responses to changing environmental conditions (Moritz, 1994; Castillo Vardaro, Epps, Frable, & Ray, 2018; Camacho-Sanchez et al. 2018). This is critical given the vulnerability of tropical montane ecosystems to the impacts of global climate change (GCC) (Feeley, Stroud, & Perez, 2017; Lenoir & Svenning, 2015) and the paucity of population genetic studies in Southeast Asia (Muenchow et al., 2018).

Kinabalu National Park

Kinabalu National Park (6°09'N 116deg39'E) is a UNESCO World Heritage Site located in Sabah, Malaysia. The park comprises two major mountains, Mt Kinabalu (MK) and Mt. Tambuyukon (MT), which contain a wide range of habitats from lowland tropical forest to high elevation scrub (Kitayama, 1992). At 4,095 meters above sea level (masl), MK is the tallest mountain in the Sundaland biogeographical region. It is relatively young, having reached its present height ca. 1 million years ago (Mya) (Hall et al. 2009). Eighteen kilometers to the north of MK, the less-studied MT stands at 2,579 masl (Figure 1a). MT is older - its major uplift occurred 7–11 Mya as part of the Crocker Range (Hall et al., 2009). Although multiple studies have focused on species richness across elevational gradients in KNP (e.g. Camacho-Sanchez et al., 2019; Liew, Schilthuizen, & bin Lakim, 2010; Nor, 2001), studies on population genetic structure in this landscape are limited (Camacho-Sanchez et al., 2018).

Mountain treeshrews

Treeshrews are small-bodied mammals in the order Scandentia native to South and Southeast Asia. The most comprehensive subordinal molecular phylogeny of treeshrews to date showed that Borneo is a center of treeshrew diversity, with 9 of 20 extant species including both widespread and endemic lineages (Roberts, Lanier, Sargis, & Olson, 2011).

The mountain treeshrew is the only montane *Tupaia* species endemic to Borneo. Its distribution includes montane regions of Sabah and Sarawak (Figure 1 inset) (Payne and Phillipps 2016; Hawkins, 2019). The species diverged from its sister lineage, the ruddy treeshrew (*T. splendidula*), ca. 4–7 Mya (Roberts et al., 2011). The ruddy treeshrew and other closely related species occupy lowland forest, potentially indicating elevational niche partitioning (Han, Sheldon, & Stuebing, 2000). On MK, the mountain treeshrew's range spans from ca. 900 to 3200 masl, encompassing four vegetation zones following Kitayama (1992): lowland (<1200 masl), lower montane (1200–2350 masl), upper montane (2350–2800 masl), and subalpine (2800–3400 masl). On MT, the species spans from ca. 900 masl to the summit and three vegetation zones (Camacho-Sanchez et al., 2019; Phillipps & Phillipps, 2016). The ecology, behavior, and evolution of this species are poorly known. The most comprehensive ecological study to date was conducted by Emmons (2000), who tracked individuals for one month at Poring Hot Spring, MK (900 masl). Emmons (2000) suggests that the mountain treeshrew has small home ranges compared to other *Tupaia* species, at only 2.5 hectares.

Despite the lack of ecological information, the mountain treeshrew's broad elevational distribution permits hypothesis testing regarding the effects of environmental heterogeneity on population genetic structure.

Study objectives

Our first objective is to determine if there is significant genetic differentiation between mountain treeshrews on MT and MK, which would have implications for conservation monitoring and management of the species in KNP and beyond. Our second goal is to assess population genetic structure across the species' elevational extent on both peaks. We test the hypothesis that gene flow is restricted across the steep ecological gradient that mountain treeshrews inhabit. We predict that mountain treeshrews will exhibit significant differentiation in neutral genetic markers 1) between mountain peaks, due to limited dispersal across the lowland habitat that connects them, and 2) across elevations – with greater differentiation on MK due to its higher elevation and associated environmental variability. Our final objective is to evaluate the utility of ultraconserved element (UCE) loci for estimating population genetic parameters and structure at fine geographic scales within a species. Previous studies have shown that UCEs are sufficiently variable to resolve phylogenies on a phylogeographic scale (e.g. Harvey, Smith, Glenn, Faircloth, & Brumfield, 2016; Mason, Olvera-Vital, Lovette, & Navarro- Siguenza, 2018; Smith, Harvey, Faircloth, Glenn, & Brumfield, 2014) and to answer questions regarding recently diverged species (Oswald et al., 2016; Winker, Glenn, & Faircloth, 2018). However, based on a Web of Knowledge literature search (accessed April 12, 2020) using the keywords 'population' AND 'ultraconserved element', ours is the first study to use these markers to study population genetics at a landscape scale. We describe a modified UCE processing pipeline to generate single nucleotide polymorphism (SNP) and phased pseudo-haplotype sequence datasets.

Materials and methods

Sample collection

We trapped small mammals on MK and MT during two field seasons in 2012 and 2013. Our trapping methodology and permitting information is described in Camacho-Sanchez et al. (2019). Briefly, we set traps from ca. 331 to 2,509 masl on MT and from ca. 503 to 3,466 masl on MK (Figure 1a). The mountain treeshrew was the most frequently caught species, representing 37.5% of all catches. For this study, we included 92 *Tupaia* individuals from our collection: 84 mountain treeshrews and 8 outgroup individuals from 2 congeners, the pygmy treeshrew (*T. minor, n = 2*) and the large treeshrew (*T. tana, n = 6*) (Table S1). We also received a loan of genomic DNA from two ruddy treeshrew voucher specimens housed at the University of Michigan Museum of Zoology (UMMZ174428 and UMMZ174429).

Laboratory methods

All laboratory work was performed at the Center for Conservation Genomics (CCG), Smithsonian Conservation Biology Institute, Washington, DC. We extracted DNA from liver and ear punch samples using a DNeasy Blood and Tissue Kit (Qiagen, Valencia CA) following the manufacturer's protocol. We amplified whole mitogenomes in two fragments using long range PCR, fragmented the PCR products to an average length of 500 base pairs (bps) using a Qsonica Q800R sonicator (QSonica, Newtown, CT, USA), and prepared single-indexed DNA libraries for sequencing using a Kapa LTP Library Preparation kit (Kapa Biosystems, Wilmington, MA) following Hawkins et al. (2016). We pooled libraries equimolarly and sequenced on an Illumina MiSeq with 2 x 100 base pair (bp) reads (Illumina, Inc., San Diego, CA).

We used in-solution DNA hybridization to enrich genomic DNA for UCEs following Hawkins et al. (2016). We sheared DNA extracts and constructed indexed libraries as above. We quantified libraries using a Qubit? fluorometer (Life Technologies) with a 1x dsDNA HS assay kit and multiplexed 4-8 samples equimolarly prior to enrichment. We used a NimbleGen SeqCap EZ? kit (Roche, Basel, Switzerland) containing 54,689 unique 60-bp DNA probes representing 5,561 vertebrate UCE loci with an average of 4x tiling per base per locus to enrich multiplexed libraries following the manufacturer's protocol. Post-enrichment libraries were amplified with 12–14 cycles of PCR using Kapa HiFi HotStart DNA polymerase (Kapa Biosystems, Wilmington, MA) following the manufacturer's protocol.

Following visualization on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) with High Sensitivity DNA kits, enriched libraries were quantified via qPCR using the Kapa Biosystems Illumina Library Quantification Kit (Kapa Biosystems, Wilmington, MA). Samples were pooled equimolarly and sequenced with 2 x 150 bp reads on Illumina HiSeq2000 (Semel Institute of Neurosciences, UCLA, & University of Copenhagen, Denmark) and MiSeq? platforms (CCG).

Data analysis: Mitogenomes

Assembly and alignment

Mitogenome amplicon reads were quality filtered with Trimmomatic v.0.33 (Bolger, Lohse, and Usadel 2014) with parameters SLIDINGWINDOW: 4:15 and MINLEN: 36. Since the only publicly available mitogenome representing any *Tupaia* species (the northern treeshrew, *T. belangeri*NC_002521; Schmitz, Ohme, & Zischler, 2000) is highly divergent from our study species (Roberts et al., 2011), we first generated reference mitogenomes for the mountain treeshrew and 3 more closely related outgroup species: the pygmy treeshrew, large treeshrew, and ruddy treeshrew. For each species, we selected one individual with the highest number of sequencing reads (pygmy treeshrew, BOR 443; large treeeshrew, BOR 010, & ruddy treeshrew, UMMZ174429) and assembled sequences*de novo* using the MIRA v1.0.1 plugin in Geneious v9.1.2 (Biomatters Ltd.). Quality filtered sequence reads were mapped to the appropriate reference using BWA-MEM v0.7.10 (Li 2013) with default parameters. We also assembled mitogenomes from UCE-enriched library sequences (Supplemental Information). Consensus sequences were generated with Geneious (lowest coverage to call a base 5x, and Highest Total Quality parameters) and aligned with the MAFFT v7.450 plugin (Katoh et al. 2002). We transferred annotations from the northern treeshrew reference to the consensus sequences. To rule out the presence of nuclear copies of mitochondrial genes (NUMTs), we translated all protein-coding genes to check for frame shifts or stop codons.

Genetic diversity and population structure

Because the inclusion of close relatives can bias estimates of genetic diversity and structure (Goldberg & Waits, 2010), we removed sequences representing first-order relatives identified by our SNP dataset (methods described below) and performed all subsequent mitogenome analyses with the reduced data, which we refer to as the "unrelated dataset". We defined haplotypes and calculated haplotype diversity (H_{d}), nucleotide diversity (π), and Tajima's D using DNAsp (Librado & Rozas 2009). We estimated the differentiation between MK and MT and between high and low elevations within each peak through analysis of molecular variance (AMOVA) in Arlequin v3.5 (Excoffier & Lischer, 2010) with a permutation test of 10,000 replicates to assess statistical significance. We visualized relationships among haplotypes by generating a median-joining network with the PopART software (Leigh & Bryant, 2015).

Phylogenetic analysis and modeling demographic history

We performed phylogenetic analyses to place the mitochondrial lineages detected in our mountain treeshrew samples within an evolutionary framework with respect to other Bornean treeshrew species (i.e. the large treeshrew, pygmy treeshrew, and ruddy treeshrew). We used PartitionFinder v2.0 (Lanfear et al. 2012) to select partitions and substitution models (Table S2) and estimated a phylogeny using MrBayes v3.2.6 (Ronquist & Huelsenbeck 2003). We then used BEAST v.1.8.4 (Drummond & Rambaut 2007) to estimate the timing of divergence between the mountain treeshrew mitochondrial lineages we identified.

To infer demographic history, we performed a Bayesian coalescent skyline plot analysis using BEAST v2.0 (Bouckaert et al. 2014). We used a time to most recent common ancestor (TMRCA) prior of 450,000 years before present (lognormal distribution, $\mu = 0.45$, $\sigma = 0.2$), the estimated date of divergence between the two mitochondrial lineages as determined by the dating analysis performed in BEAST. Analytical details are provided in Supplemental Information.

SNPs within UCEs

Genotyping SNPs

To generate the SNP dataset, we followed the PHYLUCE v.1.5.0 pipeline with default parameters (Faircloth, 2016) for sequence trimming,*de novo* assembly of contigs, identification of UCE loci, and sequence alignment. We generated a pseudo-genomic reference by aligning each locus with MAFFT v7.407 and trimming using Gblocks v0.91b with default parameters (Castresana, 2000). We then used Geneious v9.1.2 to generate a consensus sequence for each locus, replacing ambiguity codes with an appropriate nucleotide at random. We used Picard v1.106 (http://broadinstitute.github.io/picard/), and SAMtools v1.9 (Li et al., 2009) to create sequence dictionaries and reference indices from the reference. We used the PHYLUCE script *snps.py* to automate alignment of trimmed reads from each sample to the reference with BWA-MEM v0.7.17, and then called SNPs with the HaplotypeCaller tool of the Genome Analysis Toolkit v3.7 (McKenna et al., 2010) following Giarla and Esselstyn (2015). Using VCFtools v0.1.14 (Danecek et al., 2011), we removed SNPs that failed to pass GATK quality filters (QD < 2.0 || FS > 60.0 || MQ < 40.0 || HaplotypeScore > 13.0 || MappingQualityRankSum < -12.5 || ReadPosRankSum < -8.0), and selected SNPs with a minimum depth of coverage of 8 per individual and a minor allele frequency [?] 5%. We used *HD_plot.py* (McKinney, Waples, Seeb, & Seeb, 2017) to filter SNPs resulting from putative paralogs or wrongly assembled contigs from the dataset by removing SNPs with heterozygosity > 0.75 and a read-ratio deviation score D >10. The D statistic is a measure of deviation from the expected allelic read ratio of 1:1 when reads are summed over all heterozygous individuals. This method more accurately identifies true SNP loci than methods relying on read depth or heterozygote excess alone (McKinney et al., 2017). After filtering with *HD_plot.py* , we removed SNPs that were out of Hardy-Weinberg Equilibrium after Bonferroni correction for multiple comparisons ($p < 10^{-5}$) using VCFtools v0.1.16. To generate a set of unlinked SNPs, we selected a single SNP per UCE using VCFtools v0.1.16 '-thin 2000'. We used the unlinked SNP dataset with 10% missing data for all SNP-based analyses except calculation of genetic diversity and effective population size, Principal Components Analysis (PCA), Discriminant Analysis of Principal Components (DAPC) and Bayesian cluster analysis with STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000), for which we used a dataset with no missing data.

Generating phased pseudo-haplotype sequences

In order to include multiple SNPs per UCE locus as well as invariant sites, we generated multiple sequence alignments of pseudo-haplotypes. We did this by using the EMIT_ALL_SITES output mode of the GATK HaplotypeCaller tool. We filtered the resulting VCF file to include only UCE loci with at least one SNP with no more than 10% missing data. We then generated alignments from the VCF file with a custom Ruby script,*vcf2aln* v0.4.2 (https://github.com/campanam/vcf2aln, Supplemental Information). This script utilizes phasing information where present and randomly selects an allele where phase is unresolved.

We trimmed aligned, phased UCE sequences with Gblocks Version 0.91b (Castresana, 2000) using default parameters and quantified informative sites with the *phyluce_align_get_informative_sites.py* PHYLUCE script. For the final dataset, we retained loci with more than one and fewer than 10 parsimony informative sites (PIS). We removed loci with more than 10 PIS because these are likely the result of assembly or alignment errors.

Genetic diversity and effective population size

We removed individuals that were identified as first-degree relatives (parent-offspring or full siblings) according to the KING v2.1.4 software (Manichaikul et al., 2010), i.e. those with kinship coefficients [?] 0.18. Using the unlinked SNPs with 10% missing data, we first calculated pairwise kinship values and identified putative family groups with the KING software and then ran a PC-AiR analysis with GENESIS v2.2.2 (Conomos, Reiner, Weir & Thornton, 2016) in R (R Core Team 2017, applies to all subsequent use of R) to identify an "unrelated" subset of individuals. We used GenAlEx v6.503 to estimate the power of the SNP dataset to differentiate individuals by calculatingP_{IDsib} , the probability of two individuals having identical genotypes assuming siblings are present in the data (Waits, Luikart, & Taberlet, 2001).

To estimate genetic diversity, we calculated average expected and observed heterozygosity (H_e andH_o) and the inbreeding coefficient (F_{IS}) with VCFtools v0.1.16 using our SNPs with no missing data. We also

concatenated FASTA alignments of UCE sequence pseudo-haplotypes end-to-end for all individuals in the unrelated dataset and used the maximum composite likelihood method to calculate nucleotide diversity (π) in MEGA v7.0.26 (Kumar, Stecher, & Tamura, 2016).

We estimated effective population sizes (N_e) using the linkage disequilibrium model with random mating (Waples & Do, 2008) implemented in NeEstimator v2.1 (Do et al., 2014). We report estimated N_e values using 'Lowest Allele Frequency Used' 5% and 95% confidence intervals generated by the 'Parametric method' for unrelated individuals for each population cluster identified by STRUCTURE separately.

Population structure

We characterized population genetic structure using the SNP dataset with no missing data. We performed PCA and DAPC with the Adegenet 2.1.1 package (Jombart, 2008) in R. For the DAPC analysis, we first conducted$K$ -means clustering and selected the number of clusters based on the lowest Bayesian Information Criterion (BIC) value. We performed cross-validation with the function xvalDapc to determine the number of PCs to retain by calculating the lowest root mean squared error. We then ran DAPC, retaining 20 PCs and 2 discriminant functions.

We used the Bayesian clustering algorithm in the program STRUCTURE v2.3.4 to infer the number of population clusters (K) and the proportion of individual membership assigned to each cluster (q_k). We used a burn-in of 500,000 steps followed by 1,000,000 recorded steps, tracking the probability of the data given$K$ (LnP(D)) to ensure that we ran the program long enough for the values to stabilize. We used the admixture model, no location priors, and assumed correlated allele frequencies (Falush, Stephens, & Pritchard, 2003). We performed a simulation with K from 1 to 7 with 10 replicates each and identified meaningful K values using the ΔK method (Evanno, Regnaut, & Goutdet, 2005) implemented in STRUCTURE HARVESTER v0.6.94 (Earl & vonHoldt, 2012). We combined replicate runs using CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007).

Using the SNP dataset with 10% missing data, we performed an AMOVA and calculated pairwise F_{ST} values between populations identified by STRUCTURE in GenAlEx v6.5 (Peakall & Smouse, 2012), with 10,000 permutations to generate the null distribution. To investigate local spatial structure, we performed a Mantel test using ade4 v1.7 (Dray & Dufour, 2007) in R on both the full and unrelated dataset. We tested for a correlation between pairwise genotypic distance and Euclidean geographic distance with 9,999 permutations to generate the null distribution. We also generated a Mantel correlogram to test for spatial autocorrelation between pairs of treeshrews at different distance classes using GenAlEx. We first calculated pairwise linear geographic and genotypic distances, and then used the 'Spatial' option with 9,999 permutations. We defined 7 distance classes (0.2, 1.0, 2.0, 5.0, 10.0, 15.0, and 18.0 km) based on Sturges's Rule (Sturges, 1926), chosen to ensure sufficient comparisons within each class. Finally, we calculated the average, median, and maximum geographic distances between pairs of individuals in each kinship class corresponding to first, second, third-order, and distant relatives (Table 1). To test for significant differences between the means in each kinship class, we performed a one-way ANOVA in R with a Tukey Honest Significant Differences test and a Bonferroni correction for multiple comparisons (Combs, Puckett, Richardson, Mims, & Munshi-South, 2018).

Modeling migration

We used the program MIGRATE-N v3.6 with its Bayesian implementation (Beerli, 2005) to compare support for six different models of population structure and migration (Figure 2). We used the phased pseudo-haplotype sequence dataset for this analysis to take advantage of the higher information content in DNA sequences relative to SNPs. Although our workflow may generate chimeric sequences in instances where phase is unresolved, we do not expect that this would affect model selection (Andermann et al., 2018; Beerli *pers. comm.*). To ensure that phase did not affect model inference, we ran the MIGRATE-N analysis twice with different configurations of variants within haplotypes while maintaining all other settings.

We compared the following models: 1) panmixia, 2) four populations (high elevation MK, low elevation

MK, low elevation MT and high elevation MT) with bidirectional migration between adjacent pairs, 3) three populations (high elevation MT, low elevation MT and all of MK) with bidirectional migration between adjacent pairs, 4) three populations with migration between all pairs, 5) two populations (high elevation MT separate from all others) with bidirectional migration, and 6) two populations with unidirectional migration from high elevation MT (Figure 2).

For model 2, we assigned individuals to populations based on their sampling location: low elevation individuals $<$ 2000 masl, and high elevation [?] 2000 masl. For models 3, 4, 5, and 6, we assigned individuals based on STRUCTURE output for $K = 3$ and $K = 2$, respectively. We randomly selected five individuals from each population cluster ($n = 10$ haplotypes). We did not include all individuals because for coalescent processes, increasing the sample size above this does not necessarily improve accuracy, but substantially increases computation time (Felsenstein, 2005). For each model, we ran two long chains of 20,000,000 steps, sampled every 100 steps with 50,000 steps per chain discarded as burn-in, and with four heated chains. To ensure comparability across models, we ran the most complex model first and used the same prior distributions and run parameters for all subsequent models. We assessed chain mixing through acceptance ratios and ESS of parameters and genealogies. We calculated log Bayes factors (LBF) and model probability using the Bezier approximation of the marginal model likelihood and the formula described in (Beerli & Palczewski, 2010).

Results

DNA Sequencing

We obtained mitochondrial genome sequences from 83 mountain treeshrew individuals and 10 sequences from three congeners including the large treeshrew ($n = 6$), the small treeshrew ($n = 2$) and the ruddy treeshrew ($n = 2$). Mitogenomes were sequenced to an average depth of 50×. Sequences were submitted to GenBank (Accession numbers pending).

We sequenced UCEs from 80 mountain treeshrews (NCBI SRA number pending). Each UCE-enriched library was sequenced with a mean of 2.3 million reads (914,104–7,011,836), yielding a mean of 3,344 UCE loci (2,137–3,489) per sample. The total number of UCE alignments that we used to generate the pseudo-reference was 4,106, and the mean length was 495 base pairs (149–2167 bps). After aligning reads to the pseudo-reference and quality filtering, there were 7,861 SNPs including multiple SNPs per locus. After removing loci with more than 10% missing data across individuals, 3,168 SNPs remained. The unlinked SNP dataset included 1,794 independent SNPs. Removing loci with missing data left 684 unlinked loci. In the phased pseudo-haplotype sequence alignment dataset, after removing loci with less than one (114 loci) and more than 10 PIS (16 loci), 1,664 UCE alignments remained (Figure S1).

Kinship

Pairwise kinship calculations revealed several groups of putatively related individuals. After removing first-order relatives ($n = 22$ with kinship [?] 0.18) from the dataset, 58 individuals remained, including 33 from MT and 25 from MK.

Mitogenomes: genetic diversity, population structure, and demographic inference

There were 36 unique mitochondrial haplotypes in the dataset that included close relatives ($n = 83$), and 34 among the 58 "unrelated" individuals. All subsequent analyses were performed with the unrelated dataset. H_{d} was 0.977 (SD 0.008), and π was 0.00583 (SD 0.0006). Phylogenetic analyses recovered mountain treeshrews as a monophyletic group with two deeply divergent but sympatric lineages. The average number of nucleotide substitutions per site between the two lineages is 0.013 (Figure S2). Outgroup relationships were consistent with the topology in Roberts et al. (2011). The BEAST dating analysis suggests that the lineages diverged ca. 450,000 ybp (95% Highest Posterior Density, HPD, 346,000–631,900 ybp) (Figure S3).

The median joining haplotype network (Figure 3) shows that the two mountain treeshrew haplogroups are sympatric on both MT and MK. Three haplotypes are found on both mountains (Table S3). Including

related individuals, haplogroups 1 and 2 are found in near equal proportion on MK and MT (16 and 14 individuals, respectively), while haplogroup 1 is more frequent on MT (46 out of 53 individuals) (Figure 4a). The AMOVA on the unrelated dataset showed significant differentiation between the two mountains ($F_{ST} = 0.133$, $p = .00812$), with 13.3% of variance accounted for by differences between mountains and 86.7% within mountains. Dividing the population into high ([?] 2000 masl) and low (< 2000 masl) elevation groups on each peak, 90.42% of the total variance is accounted for by within-group variation, and 9.58% among ($F_{ST} = 0.096$, $p = 0.027$). Pairwise comparisons showed significant differences between high elevation MK and low elevation MT ($F_{ST} = 0.15, p = 0.023$) and high elevation MK and high elevation MT ($F_{ST} = 0.18$, $p = 0.013$).

Tajima's D test was not significant, indicating a lack of evidence for recent population contraction or expansion. In the Bayesian skyline plot analysis, the 95% HPD of the population change parameter included zero, meaning that we cannot reject the hypothesis of zero demographic changes in the last 60,000 years.

SNPs: Genetic diversity

The nucleotide diversity of phased UCE pseudo-haplotypes (1,664 alignments concatenated end-to-end) for all 58 unrelated mountain treeshrews was 0.001732 (SE 0.000022). Using the SNP dataset with no missing data, average individual heterozygosity for all 80 individuals was 0.2297 (SD 0.027), and the average inbreeding coefficient (F_{IS}) was 0.0121 (SD 0.12). For the 58 unrelated individuals, average heterozygosity was 0.2260 (SD 0.027), andF_{IS} was 0.0187 (SD 0.12). Bartlett's test revealed that the variances in observed and expected heterozygosity were not significantly different ($K^2 = 1.676, p = 0.195$). Average F_{IS} was higher on MK than MT, but the difference was not significant (0.04 and 0.01 respectively, Welch two-sample t-test $p = 0.2$). Using the dataset with 10% missing data, the probability of two individuals having identical genotypes assuming siblings are present (P_{IDsib}) was 1.54×10^{-199} .

Population Structure

Both DAPC and STRUCTURE indicated that the most likely number of population clusters was two and the second most likely was three, as determined by BIC and the ΔK method, respectively. The ΔKmethod is biased toward $K = 2$ (Janes et al., 2017; Campana, Hunt, Jones, & White, 2011) and simulation studies have shown that the mean probability (MeanLnP(K)) output from STRUCTURE performs better in scenarios with high gene flow and low F_{ST} (Latch, Dharmarajan, Glaubitz, & Rhodes, 2006). Because $K = 3$ produced the highest MeanLnP(K) in the STRUCTURE analysis (Table S4a), we consider this a relevant model and show the proportion of individual membership in each cluster as defined by each of the two analyses for both $K = 2$ and $K = 3$ (Figure 5). We also ran STRUCTURE separately for individuals caught on MK and MT, with settings described above except we ran simulations for $K = 1$–5. We found no evidence of structure among MK individuals. MT individuals were divided into two clusters - one with individuals [?] 2000 masl and one with individuals < 2000 masl, with individuals of mixed ancestry at 2000 masl (Table S4b).

Cluster membership is mostly concordant between the DAPC and STRUCTURE analyses, except STRUCTURE assigned mixed ancestry to many individuals while DAPC did not. This is not unexpected as previous studies have shown that DAPC may underestimate admixture (Frosch et al., 2014) while STRUCTURE is more accurate at assigning mixed ancestry (Bohling, Adams & Waits, 2012). When $K = 2$, individuals at 2000 and 2400 masl on MT form a separate cluster from low elevation MT + MK (Figures 4 & 5), with mixed ancestry individuals at 2000 masl MT. For $K = 3$, the divisions are between high elevation MT, low elevation MT, and MK, with individuals assigned mixed ancestry at low elevation MK (900 masl) and 2000 masl MT (Figure 5).

The PCA shows a similar pattern. Eigenvector 1 (7% variation explained) separates the two mountains, with overlap among individuals at 900 masl. Eigenvector 2 (4% variation explained) partially separates individuals by elevation, with lower elevation individuals clustered together (Figure 6). With $K = 2$, after removing individuals that could not be assigned to a STRUCTURE cluster (cutoff $q_k$value < 0.6), F_{ST} is 0.05 ($p = 0.0001$). The AMOVA showed 95% of variation is partitioned within clusters and 5% between. With $K = 3$, removing individuals withq_k values < 0.6,F_{ST} between MK and low elevation MT was 0.035

($p = 0.001$), between MK and high elevation MT 0.092 ($p = 0.0001$), and between low elevation MT and high elevation MT$F_{ST} = 0.065$ ($p = 0.0005$) (Tables 2a & 2b). The AMOVA showed that 94% of variation is distributed within clusters, and 6% among.

Including data for all 80 individuals, the Mantel test revealed a significant, positive correlation between genotypic distance and geographic distance ($r = 0.287$, $p = 0.0001$). Including the 58 unrelated individuals, the correlation was weaker but statistically significant ($r = 0.05$, $p < 0.0001$). The correlogram showed significant positive autocorrelation between individuals at distances of 200 m and less ($r = 0.091$, pr -rand [?] p r -data = 0.0001) and 200 m–1 km ($r = 0.036$, p r -rand [?] p r -data = 0.0001); autocorrelation was no longer significant at 2 km (r = -0.001, p r -rand [?] p r -data =0.598). At subsequent distance classes (5, 10, 15, and 18 km), individuals have greater genetic distance than expected at random; i.e., there is a significant negative correlation between genetic and geographic distance (p r -rand [?] r- data = 0.009, 0.0001, 0.0001, 0.0001, respectively) (Figure S4). The average geographic distance between pairs of first-order relatives was 162.5 m, second order was 1.2 km, third order was 4.8 km, and between distant or 'unrelated' individuals was 12 km (Table 1). Differences between first and third, first and distant, second and distant, and third and distant relatives were significant ($p < 0.05$).

Population and migration models

Model 4 (Figure 2) was the best fit model as determined by Bayes Factors in the MIGRATE-N analysis (Table S5). This result was consistent across both runs of the program with alternative phasing. Model 4 divided the population into three groups: high elevation MT, low elevation MT, and MK, with high rates of bidirectional migration between all pairs. The next best model was model 5, which divides the population into high elevation MT and low elevation MT + MK with bidirectional migration (Figure 2). Across models, the mean migration rate from high elevation MT to MK was greater than from MK to MT (1.3–19×, Table S6).

The results from NeEstimator suggest a larger effective population size on MT (< 2000 masl + [?] 2000 masl) than MK despite the smaller available habitat on MT (250 vs. 125, respectively) (Table 2 and Supplemental Information).

Discussion

High gene flow across a heterogeneous landscape

Seasonally stable temperatures and steep ecological gradients on tropical mountains are predicted to limit dispersal across elevations (Janzen, 1967; Ghalambor et al., 2006). This prediction is supported by several studies that show limited gene flow and significant genetic differentiation across elevations in taxa with continuous elevational distributions (Bertrand et al., 2014; Mila, Warren, Heeb & Thebaud, 2010; Gadek et al., 2018), sometimes with elevational parapatry between sister species (Linck et al., 2019; Raxworthy et al. 2008a; DuBay & Witt, 2014). For example, Gadek et al. (2018) found cryptic population genetic structure differentiating high from low elevation populations in 3 of 4 Peruvian songbird species with continuous elevational distributions.

Our results are not consistent with a hypothesis of restricted gene flow across elevational gradients. We report evidence of high gene flow between MK and MT as well as between low and high elevations on both peaks, indicating that neither the lowland habitat connecting the two peaks, nor the steep elevational gradient across which mountain treeshrews occur on each peak, has significantly limited effective dispersal.

The mountain treeshrew population in KNP is best described as comprising two or three clusters. The primary population division does not correspond to the two peaks; rather, the summit region of MT ([?] 2000 masl) is distinct (Figure 5a). When dividing the population into three clusters, individuals at low elevation MK show mixed ancestry with low elevation MT (Figure 5b). Additionally, the MIGRATE-N analysis supports the division of individuals into three population clusters (high elevation MT, low elevation MT, and all of MK), with high migration rates between all pairs (Figure 2).

The temperature lapse rate in KNP is -0.55degC per 100 meters of elevation gain (Kitayama, 1992) which means that mountain treeshrews experience a 12.65degC average range in temperature between 900 and 3200 masl on MK. This temperature range is higher than the thermal neutral zone for most mammals that weigh < 1 kg (Khaliq, Hof, Prinzinger, Bohning-Gaese, & Pfenninger, 2014). On MT, mountain treeshrews experience an 8.8degC range from 900 to 2500 masl (Camacho-Sanchez et al., 2018). On MK, the species occupies four distinct vegetation zones, while on MT it occupies three (Kitayama, 1992). If gene flow were restricted due to limited elevational dispersal or selection against cross-elevation migrants, we would expect to find greater genetic differentiation on MK because of the broader elevational range and associated diversity of environmental factors on this slope compared to MT. However, the summit of MT was consistently recovered as the most distinct population cluster while individuals caught along the entire elevational gradient on MK form a single cluster (Figures 4 & 5). This suggests that elevation is not the primary variable influencing genetic structure in KNP.

Tropical montane species tend to have narrower ecological tolerance and elevational ranges than those in the temperate zone. McCain (2009) reviewed 170 montane gradients across vertebrate taxa to test this hypothesis and found that most groups showed the predicted pattern of decreasing range size with decreasing latitude. However, in rodents there was either no relationship or range size increased with declining latitude. This finding could be explained by the presence of cryptic species pairs or genetic differentiation separating low elevation populations from high (den Tex, Thorington, Maldonado, & Leonard, 2010; Hinckley, Hawkins, Achmadi, Maldonado, & Leonard, in review). However, McCain (2009) hypothesized that rodents may be able to cope with the lower temperatures associated with increasing altitude through behavioral adaptations including burrowing. Further studies are necessary to determine whether mountain treeshrews show behavioral or phenotypic plasticity, adaptive phenotypic (e.g. Branch et al., 2017) or genetic differentiation despite gene flow, or a generalist phenotype (e.g. Feijo et al., 2019) that allows them to persist across such broad environmental conditions which is rare among small mammals in this landscape (Nor, 2001; Camacho-Sanchez et al., 2019).

Population genetic structure shaped by isolation-by-distance and historical dynamics

The population genetic pattern that we observe is partly consistent with isolation-by-distance (IBD). The Mantel test showed a significant, positive correlation between pairwise geographic distance and genetic distance although the effect size is very small when individuals with high kinship values are removed from the dataset. Individuals located within 1 km have significantly lower pairwise genetic distances than expected by chance, and this significance drops off by 2 km (Figure S4). Additionally, pairwise F_{ST} between the non-adjacent MT summit and MK clusters is greater than the value between neighboring clusters (Table 2a). High rates of gene flow between adjacent demes across the landscape, with relatively short dispersal distances as suggested by the correlogram and ANOVA (Figure S4, Table 1), could have generated the clinal pattern we observe (Figure 5); however, this does not explain the distinctiveness of the summit MT cluster. The Euclidean distance between the lowest and highest sampled points on MK (ca. 13.5 km) is greater than the distance between the lowest and highest sampling points on MT (ca. 4.5 km), yet there is more population genetic differentiation on MT. This indicates that the structure we observe is not due to isolation-by-distance or isolation-by-elevation alone, and that genetic similarity decays with geographic distance at unequal rates in this landscape (Figure S5a).

Historical population dynamics, in addition to IBD, likely contributed to the observed population genetic structure. Without data from other locations in Borneo, it is difficult to determine what process(es) generated the pattern. However, we suggest a plausible scenario given known information about the relative ages of MT and MK and the degree of divergence between the mountain treeshrew and its sister species.MT reached its current elevation earlier (ca. 11–7 Mya) than MK (ca. 1 Mya) (Collenette, 1964; Hall, 1998; Liew et al. 2010). This suggests that MT was available for colonization prior to the split of mountain treeshrews from ruddy treeshrews ca. 4 Mya (Roberts et al., 2011). If mountain treeshrews were resident on MT prior to the major uplift of MK, and a second colonization event occurred later, this would explain the signature of two population clusters. We find higher-than-average genetic diversity among individuals at high elevation MT

despite its smaller habitat area (Figures 1a & S5b), which is consistent with our hypothesis that this region maintained a relatively stable, or recently reduced, effective population size over time relative to MK. Lower gene flow upslope to high elevation MT relative to gene flow towards MK may have preserved the signature of this cluster.

In the MIGRATE-N analysis, the estimated rate of migration upslope to high elevation MT was lower than migration downslope and toward MK (Table S6). We suggest that reduced gene flow to high elevation MT may be related to shifts in the plant community that occur between 1450 masl and the summit (van der Ent, Cardace, Tibbett, & Echevarria, 2018). Supporting this hypothesis, trapping success of mountain treeshrews and other small mammals is low from 1500 to 1800 masl, and increases above 2000 masl (Camacho-Sanchez et al., 2019).

By contrast, lack of differentiation across MK could have been influenced by an upslope shift at the mountain treeshrew's upper elevational limit, enabled by climate warming and upslope shifts in montane forest since the Last Glacial Maximum (LGM). During late Quaternary glacial cycles, MK's summit alternated between ice-free (during glacial minima) and ice-covered (during glacial maxima) periods (Hall et al., 2008). After the LGM ca. 20,000 ybp, the ice began to melt and by 9,200 ybp the summit was likely ice-free (Hall et al., 2008). Upslope shifts in montane forest during this period of warming could have enabled range expansion at high elevations by mountain treeshrews. Mountain treeshrews on MT likely did not experience a concurrent upslope range shift since MT has a much lower summit than MK which was never covered in ice (Hall et al., 2008). The lack of a population expansion signature in the mountain treeshrew mitogenome data could be explained by unrestricted gene flow between adjacent areas during expansion (Pierce, Gutierrez, Rice, & Pfennig, 2017). As predicted for a recent expansion, we find lower-than-average genetic diversity among high elevation MK individuals ([?]1600 masl) using estimated effective migration surface modeling (Petkova, Novembre, & Stephens, 2016) to visualize genetic diversity on the landscape (Figure S5b).

Mito-nuclear discordance

The population genetic pattern inferred from our mitogenome data is discordant with the nuclear SNP dataset, although it is not inconsistent with a scenario of two colonization events to KNP. Phylogenetic analyses revealed two divergent mitochondrial lineages within mountain treeshrews; both lineages are found on both mountains, but haplogroups 1 and 2 are equally represented on MK while haplogroup 1 is more frequent on MT (Figures 3 & 4). As mentioned above, MT provided montane habitat earlier than MK. If KNP were colonized a second time by mountain treeshrews from another part of their range, such as the southern portion of the Crocker Range, this would explain the presence of two sympatric divergent lineages within Kinabalu Park. The greater frequency of haplogroup 2 on MK could be explained by the closer geographic proximity of MK to the Crocker Range (Figure 7) combined with male-biased dispersal limiting the movement of haplogroup 2 from MK to MT. Lack of recombination in the mitochondrial genome would have retained the divergence between these the two lineages whereas recombination in nuclear SNPs would result in genetic admixture between the two groups. However, as noted above, the cluster on high elevation MT has maintained a moderate level of differentiation.

As an alternative explanation, this pattern could have been caused by a single colonization event of two sympatric lineages that diverged elsewhere in Borneo, for example, due to isolation in interglacial refugia and mixing during glacial maxima when montane forest was at its maximum extent (Cannon, Morley, & Bush, 2009; den Tex et al, 2010). However, this scenario implies that the colonization of KNP by mountain treeshrews would have occurred after the divergence between the two lineages ca. 450,000 ybp, which is relatively recent compared to the age of MT (at least 7 million years) and the age of the species (ca. 4 million years). Multiple colonization events to MK have been inferred in other taxa, including plants in the genus *Rhododendron* (Merckx et al., 2015).

Gawin et al. (2014) documented a similar pattern in mountain blackeyes (*Chlorocharis emiliae*) in Borneo; they found two divergent mitochondrial haplogroups on MK, with one lineage sister to a lineage found on Mt. Trus Madi, a mountain south of MK within the Crocker Range (Figure 7). The pattern inferred from

SNP data in a subsequent study was not concordant, with a single lineage found on MK (Manthey et al., 2017). This similar pattern may indicate a common colonization history between mountain blackeyes and mountain treeeshrews. Future studies should include broader geographic sampling of mountain treeshrews, including individuals from across the Crocker Range, to test the hypothesis of multiple colonization events and to determine the phylogeographic history of this species in Borneo.

Utility of UCEs for fine-scale population genomics

Since the original design of DNA hybridization probes to capture and sequence UCEs for tetrapods (Faircloth et al., 2012), UCEs have proven capable of resolving phylogenies at multiple timescales and across taxa (e.g. Hawkins et al., 2016; McCormack et al., 2012; Smith et al., 2014; Sun et al., 2014). UCEs can resolve phylogeographic patterns, population structure, and demographic history of recently diverged species (e.g. Harvey et al., 2016; Winker et al., 2018; Zarza et al., 2016). Here, we show that sequence capture of ca. 5,000 UCEs yielded two highly informative datasets suitable for landscape-scale population genomics. These datasets were able to resolve patterns of population structure in mountain treeshrews within KNP, an area of approximately 754 km^2. The SNP dataset provided sufficient statistical power to identify individuals with high probability ($P_{IDsib} = 1.54 \times 10^{-199}$) and to identify putative family groups using pairwise kinship estimates. This suggests the potential for UCEs to be used for population genomic studies.

UCEs are valuable for studying species like the mountain treeshrew for which few genomic resources are available. Although RAD-seq methods also do not require reference genomes, UCE capture produces data with similar information content and has several benefits over RAD-seq: 1) UCEs enable direct comparison of inferences drawn from the same set of loci across species, allowing conclusions to be drawn about the effects of historical processes on diverse taxa (Lim et al., 2020), 2) UCE capture offers repeatability such that studies can compare inferences for the same species across time and geographic regions (Harvey et al., 2016), and 3) UCE capture can be performed on low-quality DNA, including DNA derived from historical museum specimens (Hawkins et al., 2016; Lim et al., 2020; Lim & Braun, 2016, Tsai et al., 2019).

Conservation Implications

Anthropogenic GCC is one of the most significant threats to Earth's biodiversity (Elsen & Tingley, 2015; Bellard et al., 2014). Tropical montane species are predicted to suffer the greatest effects because they are likely to be endemic with restricted geographic ranges and narrow climatic tolerance (Williams, Jackson, & Kutzbach, 2007; La Sorte & Jetz, 2010). Several studies have documented upslope range shifts in response to GCC-associated temperature and precipitation changes (e.g. Freeman, Scholer, Ruiz-Gutierrez, & Fitzparick, 2018; Raxworthy et al., 2008b; Chen et al., 2009). These shifts can result in reduced or fragmented habitat, decreasing genetic diversity and making species vulnerable to extinction (Moritz et al., 2008; Williams et al., 2007). In addition, in the case of locally adapted, highly structured populations, increased dispersal and gene flow upslope could introduce maladaptive genes (Weiss-Lehman & Shaw, 2019). It is therefore imperative to understand how tropical montane populations are structured in order to predict, monitor, and mitigate the effects of GCC.

Here, we provide a foundational estimate of population structure, genetic diversity, and historical gene flow for mountain treeshrews in KNP. These data can be used to monitor GCC-induced population genetic changes over time. Montane communities in KNP could experience an upslope shift of ca. 490 m by 2100 CE (Still, 1999; Camacho et al., 2018), assuming mild Intergovernmental Panel on Climate Change scenarios (IPCC 2013, www.ipcc.ch/report/ar5/wg1/). Although the factors that limit the mountain treeshrew at its lower elevational boundary are unknown, assuming that the species tracks the predicted 490 m upslope shift - whether because of climatic limitations or ecological interactions with lowland species expanding upslope - we predict that it will experience range contraction. The species already occupies the upper elevational limits within KNP, so an upslope shift in the lower bound of its distribution could not be countered with expansion at its upper limit. The lack of strong population structure across elevations means that upslope dispersal of lower elevation mountain treeshrews on MK will likely not increase extinction risk by introducing maladaptive genetic diversity. However, reduction in available habitat could make the species vulnerable.

We also predict that mountain treeshrews would maintain connectivity between MK and MT. However, the Crocker Range has few peaks above 1400 masl, and connectivity between KNP and the rest of the Crocker Range could be severed (Figure 7). This highlights the importance of KNP as a future refugium for montane species, as it contains the highest peak in the region and the greatest high-elevation forested area. Conservation efforts should focus on protecting forest habitat at 900 masl to facilitate gene flow and preserve genetic diversity.

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Data Accessibility

-DNA sequences: Genbank accessions XX-XX; NCBI SRA: XX

-UCE pseudo-haplotype multiple sequence alignments available on FigShare

Author Contributions

MTRH, MCS, and JAL organized field work; MTRH and MCS conducted field work; MTRH, JAL, JEM, and LDP designed the study; JAL, JEM, and LLR provided resources for the study; MTRH and LDP did laboratory work; LDP wrote the manuscript and performed SNP analyses; MTRH performed phylogenetic analyses; MCS contributed figures; TRW, HCL, and MGC assisted in bioinformatics; MGC and JAW developed vcf2aln; all authors participated in revisions and acceptance of the final version of the manuscript.

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Table 1. Geographic distances between pairs of individuals with different levels of estimated relatedness based on analysis with the KING software. ANOVA and Tukey Honest Significant Differences test showed significant differences in distances between all pairs except first and second-order relatives and second and third-order relatives ($p < 0.05$).

Kinship	**Relatedness**	**n**	**Average distance (m)**	**Median distance (m)**	**Max distance (m)**
> 0.18	First order	42	162.5	100.8	570.1
0.177 - 0.0884	Second order	56	1247	322.8	25850
0.0883 - 0.0442	Third order	112	4819	793.0	26490
< 0.044	Distant	2932	12310	15960	29430

Table 2. Effective population sizes and pairwiseF_{ST} of population clusters with **a)**$K = 3$ and **b)** $K = 2$. MK = Mount Kinabalu, MT = Mount Tambuyukon. N_{e} estimates are on the diagonal with 95% CI in parenthesis; F_{ST}estimates are below the diagonal, with associated p -values above the diagonal.

a .

$K = 3$	MK	MT < 2000masl	MT > 2000masl
MK (n=22)	125 (105–152)	0.00120	0.00010
MT < 2000masl (n=19)	0.035	202 (157–282)	0.00050
MT > 2000masl (n=11)	0.092	0.065	48 (40–59)

b.

$K = 2$	MK + MT < 2000masl	MT > 2000masl
MK + MT < 2000masl (n=36)	180 (160–205)	0.0001
MT > 2000masl (n=18)	0.050	57 (52–63)

Figure 1. a (left) Map of mountain treeshrew distribution (inset modified from IUCN 2019), and map of Kinabalu Park, Sabah, Borneo. Park boundaries are demarcated by dashed lines, transects by black lines, and sampling locations by white circles. Shading indicates lower and upper portion of mountain treeshrew habitat (900–2000 masl and >2000 masl, respectively) **b (right).** Image of a mountain treeshrew and a pitcher plant (*Nepenthes lowii*), Kinabalu National Park (Photo credit: Chien C. Lee). The two species have a mutualistic relationship in which mountain treeshrews feed on the sugary secretions provided by the plant and in turn provide the plant nitrogen through feces (Chin, Moran, & Clarke, 2010).

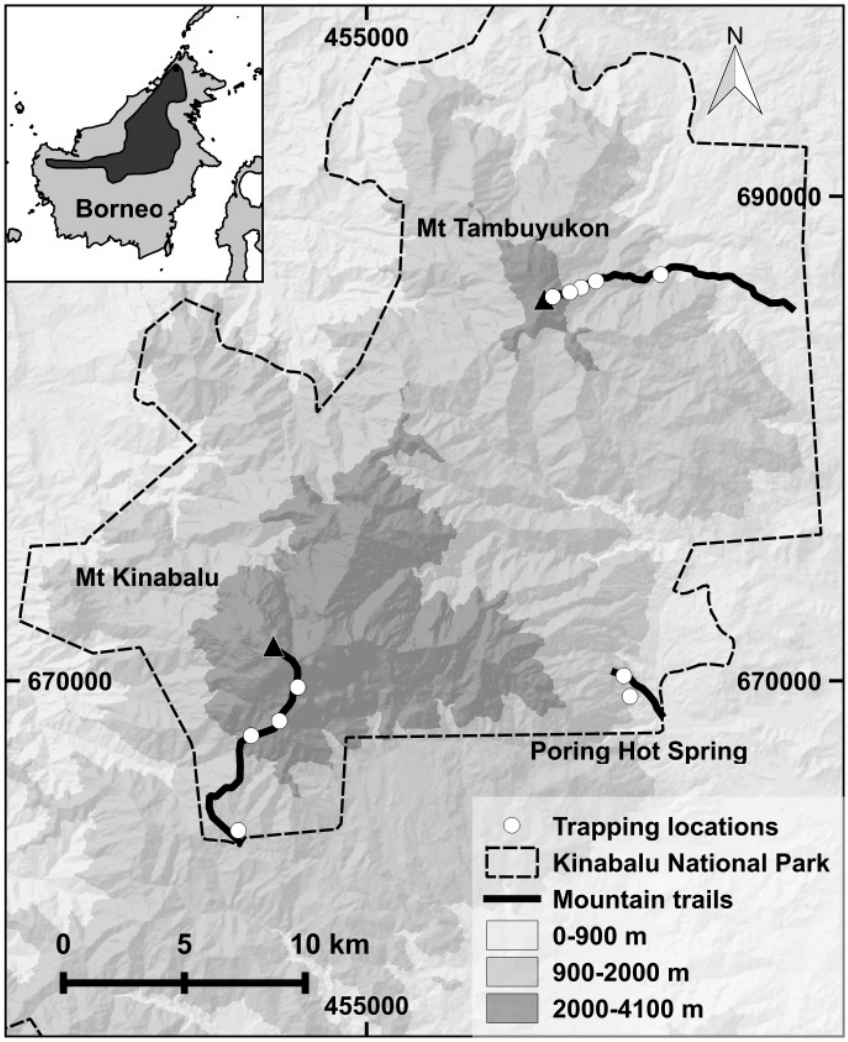

Figure 2. Population structure and migration models evaluated using MIGRATE-N. Model ranks are based on Bayes factors; log marginal likelihood values are listed in Table S4. MT, Mt. Tambuyukon; MK, Mt. Kinabalu; High [?] 2000 masl; Low < 2000 masl.

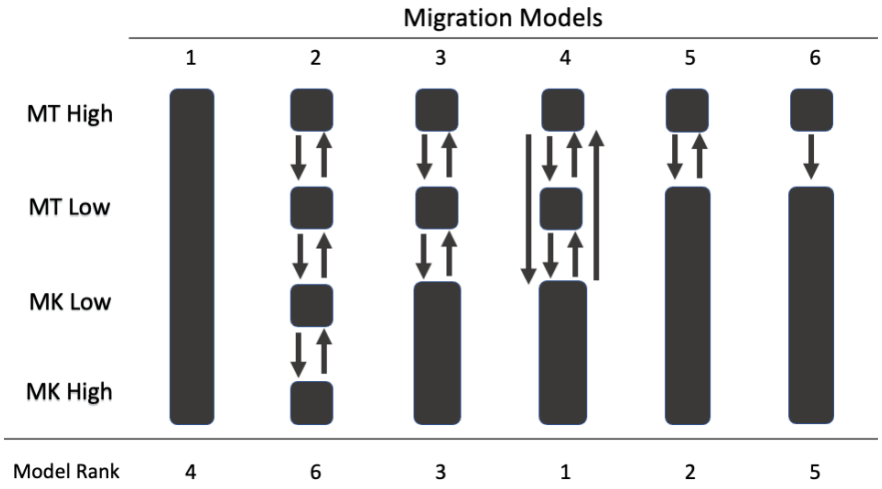

Figure 3. Median joining network of 34 mitogenome sequences. Dashed lines represent the number of base pair differences between haplotypes except in cases where the number of differences exceeds 40. Colors correspond to the two mountains (MT, orange; MK, blue). Note that the two haplogroups are not shown to scale and are separated by 186 bp substitutions.

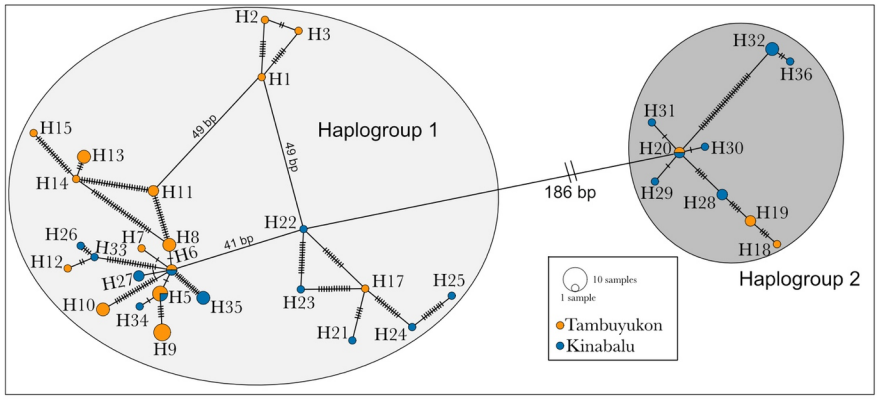

Figure 4. Elevational profiles of Mt. Kinabalu and Mt. Tambuyukon with the distribution of mitochondrial haplogroups per elevation shown below each transect line and SNP clusters above. Mitogenome pie charts indicate, for each elevation, the number of treeshrews sampled with a haplotype from mitochondrial haplogroup 1 (light grey) and 2 (dark grey). SNP pie charts indicate for each elevation the proportion of ancestry assigned to cluster 1 (light grey) and cluster 2 (dark grey).

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Figure 5. Cluster membership based on DAPC and STRUCTURE analyses for **a)** $K = 2$ and **b)** $K = 3$. Each horizontal line represents a single individual with shading showing how much of each's ancestry can be attributed to each cluster. Individuals are arranged from high elevation Mt. Tambuyukon to low elevation Mt. Tambuyukon followed by low elevation Mt. Kinabalu to high elevation Mt. Kinabalu.

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Figure 6. PCA Plot with individuals caught on Mt. Kinabalu shaded in blue and Mt. Tambuyukon in orange. PC1 explains 7% of variance; PC2 explains 4%.

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Figure 7. Estimated mountain treeshrew habitat in the year 2100 CE assuming mild IPCC scenarios. Light grey shows [?] 900 masl, which is current mountain treeshrew habitat; dark grey indicates [?] 1400 masl, which is potential mountain treeshrew habitat in 2100 assuming mild climate change as projected by IPCC. Protected areas are demarcated by dashed lines. Transects sampled in this study are shown in black, with sampling locations indicated by white circles.

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