Assessing current genetic status of the Hainan gibbon using historical and demographic baselines: implications for conservation management of species of extreme rarity

J.V. Bryant[1,2,*], D. Gottelli[1], X. Zeng[3], X. Hong[3], B.P.L. Chan[4], J.R. Fellowes[5], Y. Zhang[6,7], J. Luo[7], C. Durrant[1], T. Geissmann[8], H.J. Chatterjee[2], S.T. Turvey[1]

[1]Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK

[2]Department of Genetics, Evolution & Environment, University College London, Gower Street, London WC1E 6BT, UK

[3]Bawangling National Nature Reserve Management Office, Changjiang Lizu Autonomous County, Hainan, China

[4]Kadoorie Conservation China, Kadoorie Farm and Botanic Garden, Lam Kam Road, Tai Po, New Territories, Hong Kong

[5]The Kadoorie Institute, University of Hong Kong, Pokfulam Road, Hong Kong

[6]Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China

[7]College of Life Sciences, Yunnan University, Kunming 65009, China

[8]Anthropological Institute, University Zurich-Irchel, Winterthurerstrasse 190, Zurich CH-8057, Switzerland

*Corresponding Author: jessica.bryant@ioz.ac.uk

Abstract

Evidence-based conservation planning is crucial for informing management decisions for species of extreme rarity, but collection of robust data on genetic status or other parameters can be extremely challenging for such species. The Hainan gibbon, possibly the world's rarest mammal, consists of a single population of c.25 individuals restricted to one protected area on Hainan Island, China, and has persisted for over 30 years at exceptionally low population size. Analysis of genotypes at 11 microsatellite loci from faecal samples for 36% of the current global population and tissue samples from 62% of existing historical museum specimens demonstrates limited current genetic diversity (*Na*=2.27, *Ar*=2.24, H_e=0.43); diversity has declined since the 19th century and even further within the last 30 years, representing declines of c.30% from historical levels (*Na*=3.36, *Ar*=3.29, H_e=0.63). Significant differentiation is seen between current and historical samples (F_{ST}=0.156, *P*=0.0315), and the current population exhibits extremely small N_e (current N_e=2.16). There is evidence for both a recent population bottleneck and an earlier bottleneck, with population size already reasonably low by the late 19th century (historical N_e=1162.96). Individuals in the current population are related at the level of half- to full-siblings between social groups, and full-siblings or parent-offspring within a social group, suggesting that inbreeding is likely to increase in the future. The species' current reduced genetic diversity must be considered during conservation planning, particularly for expectations of likely population recovery, indicating that intensive, carefully planned management is essential.

Keywords: bottleneck, conservation genetics, Critically Endangered, *Nomascus hainanus*, ghost alleles

Running title: Past and present Hainan gibbon genetic status

Introduction

Conservation management decisions must be made rapidly to prevent species losses. For species of extreme rarity, which persist in single, geographically restricted populations reduced to handfuls of individuals, delays in decision-making can mean the difference between extinction and recovery (Groombridge et al. 2004, Turvey 2008, Grantham et al. 2009). As human pressures on global ecosystems intensify, more and more species are likely to decline to states of extreme rarity, making it vital to develop methods that enable identification of appropriate and/or necessary management actions for such populations.

The need for evidence-based conservation, whereby robust empirical data on ecology, population dynamics and threats are used to guide management, is now widely accepted (Sutherland et al. 2004, Segan et al. 2011). However, for extremely rare species, which are most urgently in need of management action, robust data are often unavailable and collection of new data can be extremely challenging, as the very rarity of these species makes them difficult to study. It is therefore crucial that the suitability of the evidence-based approach is evaluated for such species.

A key consideration for effective conservation of small populations is the impact that a drastic reduction in population size, or bottleneck, can have upon genetic condition. Sudden population declines can lead to concomitant losses of genetic diversity, which can in turn impact long-term viability through reduced ability to withstand environmental change (Lindsey et al. 2013), compromised disease resistance (Siddle et al. 2007), and reduced survival/reproductive fitness (Swinnerton et al. 2004, Hemmings et al. 2012). Small populations are also more vulnerable to further diversity declines through processes which have minimal impacts in larger populations, notably chance loss of alleles through magnified effects of genetic drift, and increased probability of mating between related individuals

(Frankham et al. 2009). A growing body of literature highlights the importance of contextualising information on the current genetic condition of species of extreme rarity within the context of historical patterns of genetic status and a chronology of past population dynamics, to determine the implications of genetic factors for conservation management (Groombridge et al. 2009, Raisin et al. 2012, Bristol et al. 2013).

The Hainan gibbon (*Nomascus hainanus*) (Thomas, 1892) is the world's rarest ape, rarest primate, and possibly rarest mammal species, consisting of a single population constrained to Bawangling National Nature Reserve (BNNR), Hainan Island, China (Appendix S1). Following a precipitous decline from c.2,000 individuals in the 1950s due to habitat loss and hunting (Liu et al. 1984, Zhou et al. 2005), to a reported low of only 10 individuals by the early 1990s (Zhang 1992), the species has persisted as a single remnant population for over 30 years at exceptionally low population size, with estimates since the 1980s fluctuating between 10-25 individuals (Liu et al. 1989, Zhang and Sheeran 1993, Wu et al. 2004, Zhou et al. 2005, Li et al. 2010). The current population consists of c.25 individuals in three social groups: Group A (c.11 individuals), Group B (seven individuals), and Group C (three individuals), together with a low, unknown number of solitary individuals (Turvey et al. 2015).

Despite the Hainan gibbon's Critically Endangered status, past genetic research has predominately consisted of inclusion in wider phylogenetic analyses (Su et al. 1995, Zhang 1995, Thinh et al. 2010a, b). Several authors have alluded to genetic consequences of its small population size, suggesting it may suffer from genetic constraints (Liu et al. 1989, Fellowes et al. 2008). Although the species exhibits no obvious sexual dimorphism before reproductive maturity, making it difficult to sex immature individuals visually, reports of nine of 12 offspring born between 1982-1989 being male have also led to concerns that the population exhibits an imbalanced sex ratio (Liu et al. 1989), which may limit mate

availability, social group formation and population growth (Chan et al. 2005, Li et al. 2010). There has been speculation about incest and inbreeding constraining recovery, with concern that surviving individuals are likely closely related (Liu et al. 1989, Fellowes et al. 2008). However, there has only been one attempt to investigate the species' genetic status, through assessment of diversity in the mitochondrial DNA (mtDNA) control region (Li et al. 2010). Unfortunately, methodological issues associated with this study minimise its utility in understanding the species' current genetic health, including sampling limitations (n=6 individuals, from one social group) which restrict representativeness, possible sequencing errors indicated by detection of four haplotypes in one social group, and failure to contextualise results against other gibbon species' genetic diversity or past Hainan gibbon diversity. Furthermore, the study provided no insights into fundamental demographic parameters of the current population, such as individual relatedness, inbreeding level, or offspring sex ratio. These factors, which are crucial to understanding population viability, remain unknown.

The paucity of information on genetic health of the last Hainan gibbon population precludes accurate understanding of the role this factor may play in constraining population recovery. Comprehensive assessment of the species' current genetic status within the context of its genetic history is vital to inform conservation planning. Any genetic study of a species of such extreme rarity will inevitably be limited by sample size, although for tiny populations such as that of the Hainan gibbon, sampling even a handful of animals represents a substantial proportion of the total population, and can provide important insights into demographic and population genetic status. On average, 52 species of mammals, birds, and amphibians move one threat category closer to extinction each year (Hoffmann et al. 2010), therefore studying species of extreme rarity will likely become the norm rather than the exception for

conservation managers and wildlife biologists; the challenge of assessing demographic and genetic status for such species will become increasingly commonplace, and may require assessment of the relative effectiveness of multiple metrics. There is therefore a need to understand what information can feasibly be obtained from applying current analytical approaches to datasets and sample sizes that are restricted by species rarity.

Within these constraints, we therefore aimed to quantify genetic diversity of the current Hainan gibbon population and assess whether declines in diversity have occurred through known historical population reduction. We determined present and past genetic diversity of the species and any genetic differentiation between these temporal 'populations', evaluated genetic evidence for inbreeding and past genetic bottleneck, and estimated effective population size of the current and historical populations. Empirical data on population parameters that shed light on genetic and demographic factors that may affect population recovery are also required for accurate assessment of the species' current status. We therefore also assessed key genetic characteristics of the current population, including degree of relatedness and population sex ratio (including for offspring only). This comprehensive assessment represents a new baseline for understanding the possible influence that the Hainan gibbon's current genetic condition may have on long-term population viability, and assesses the extent to which standard scientific approaches can inform conservation planning for species of extreme rarity.

Materials and Methods

Sample collection and DNA extraction

Faecal samples were collected opportunistically from the current population during fieldwork at BNNR in 2010-2011. Samples were collected immediately following observed defecations. All individuals of habituated Group B were sampled (samples B1-B7); single samples for one individual each were obtained from unhabituated Groups A and C (samples A, C). Samples were preserved by adding silica gel beads (drying-agent) to c.2-5g of scat in 15ml plastic tubes (Wasser et al. 1997, Goossens et al. 2003, Chambers et al. 2004); beads were regularly replaced upon saturation until samples were completely desiccated. Where available, additional sample material was kept in replicate silica-dried samples, with remaining material preserved in 70-90% ethanol. Samples were stored in cool dark conditions in the field and at 4°C in the laboratory. DNA was extracted using QIAamp DNA Stool Kit (QIAGEN), with minor protocol modifications to enhance removal of impurities/inhibitors and increase DNA yield, and final elution volume of 120µl to improve DNA concentration. For individuals where >1 sample was collected, multiple samples were extracted. DNA is not spread uniformly through faecal samples (Goossens et al. 2003), so where sample volume permitted, multiple independent extractions were taken to maximise probability of obtaining DNA.

Small samples (c.5x2mm) of skin, muscle or bone were obtained from 12 Hainan gibbon specimens in museum collections accessioned between 1899-1980, collected from Bawangling and elsewhere on Hainan (Appendix S2), and representing all but one of the historical specimens known at the time of this study (it was not possible to sample the holotype). DNA was extracted from skin/muscle using QIAamp DNA Micro Kit (QIAGEN) and from bone using QIAquick PCR Purification Kit (QIAGEN). A final elution volume of

100µl and post-elution addition of 5µl of 1% TWEEN (Sigma-Aldrich) were employed to increase DNA concentration.

While every precaution was taken to prevent contamination during sampling, to monitor possible human DNA contamination, hair samples (c.10 freshly-plucked hairs) were collected from all fieldworkers who collected samples and included as positive controls during DNA amplification. Blood samples from contemporary specimens of two gibbon species (*Hylobates lar*, *Nomascus concolor*: Zoological Society of London Blood and Tissue Bank) were also used as positive controls. DNA was extracted from control samples using DNeasy Blood & Tissue Kit (QIAGEN). Rigorous procedures were employed to minimise potential contamination throughout extraction, including: stringent cleaning of surfaces/equipment with 10-40% bleach and/or exposure to ultraviolet radiation; extraction of samples for different individuals on separate occasions; museum sample processing in UV-irradiated fume hood to destroy contaminant DNA; extraction of current, historical and control samples in physically separate laboratory areas in specialised facilities at Yunnan University, Kunming, China; Institute of Zoology, Zoological Society of London; and Royal Holloway, University of London.

Marker screening and genotyping

Gibbon-specific genetic markers are presently unavailable, so we amplified gibbon DNA using human-derived microsatellite primers via cross-species amplification (Goossens et al. 2000b, 2005, Vigilant and Bradley 2004). Thirty human microsatellite loci (Appendix S3) previously tested for gibbons (*Hylobates lar*, *H. muelleri*; Clisson et al. 2000, Oka and Takenaka 2001, Chambers et al. 2004, Roeder et al. 2009) and <250bp were screened; larger

184 loci are problematic when amplifying DNA from non-invasive samples (Goossens et al.
185 2000b). Loci were tested using DNA extractions from three current population samples and
186 both control gibbon samples.

187 Twenty-four microsatellite loci produced detectable PCR products for Hainan gibbon samples
188 (Appendix S3) and were used for formal genotyping. Samples were amplified using
189 fluorescently-labelled forward sequences for each primer pair via PCR in a reaction volume of
190 c.7µl containing 2µl (≤50ng) template DNA, 1.5µl (0.3µ*M*) primer, 0.02µl bovine serum
191 albumin (New England Biolabs), and 3.5µl Multiplex PCR Mix (QIAGEN, final
192 concentration 3m*M* $MgCl_2$). The thermal profile for PCR reactions consisted of: denaturation
193 and enzyme activation at 95°C (15 minutes); 30-35 cycles of denaturation at 94°C (30
194 seconds); annealing at relevant temperature (90 seconds) (Appendix S3); extension at 72°C
195 (60 seconds); final extension at 72°C (30 minutes). The 24 loci were divided into eight
196 'multiplex' mixes, each containing three loci. PCR products were visualised on an ABI
197 PRISM 3130xl Genetic Analyser (Applied Biosystems) together with GeneScan 500 LIZ Size
198 Standard (Applied Biosystems). Alleles were scored using GeneMapper V.4.1 (Applied
199 Biosystems) against the internal size standard to derive individual genotypes at each locus.

200 Consensus genotypes were derived for each current and historical sample using a multi-tube,
201 multi-sample approach and a strict set of *a priori* allele-scoring rules (Taberlet et al. 1996,
202 Goossens et al. 2000a). At least five independent PCR replicates were genotyped for each
203 extraction to minimise genotyping errors associated with low-quality template DNA (e.g.
204 false alleles, allelic dropout) (Taberlet et al. 1999); multiple extractions/sample and
205 samples/individual were genotyped where sample volume/number permitted. We calculated

the mean quality index across samples and loci to assess genotyping reliability (Miquel et al. 2006).

To ensure standardised allele sizes between samples/replicates, PCRs were prepared in physically-isolated areas to prevent cross-contamination but amplified simultaneously (same PCR), with reference samples (high-quality DNA extracted from a current sample) included in every PCR. Positive (human, gibbon) and negative controls from every stage (extraction blanks and PCR blanks) were also included during genotyping to monitor potential contamination and PCR failure. Current population sample genotypes were verified by checking for consistent allele-sharing between individuals in the current population with known parentage. Loci monomorphic for the species, and those that failed to amplify across both current and historical samples despite extensive replication, were discounted, resulting in consensus genotypes from 13 polymorphic loci (Appendix S4).

Genotyping errors due to false alleles, allelic dropout, stutter and null alleles were checked using MICRO-CHECKER V.2.2.3 (van Oosterhout et al. 2004). LOSITAN (Antao et al. 2008) was used to detect loci under selection, using 100,000 simulations and a 0.95 confidence level for neutral markers; loci falling outside this confidence interval were considered non-neutral and excluded from analysis. Between-locus linkage disequilibrium was tested in F-STAT V.2.9.3.2 (Goudet 2002).

Sex-determination

To investigate the remaining population's sex ratio, all current samples were genotyped using a fluorescently-labelled Amelogenin primer (Sullivan et al. 1993). Amelogenin amplification products have short fragment lengths (<120bp), making amplification viable for degraded

DNA (Bradley et al. 2001). PCR amplification was conducted as previously described (with T_a=55°C). Human and gibbon controls were included in all PCR replicates, and the primer was incorporated into a multiplex mix (where allele sizes in humans versus gibbons were obviously different for other primers) to ensure derived Amelogenin genotypes were from gibbon samples. PCR replication and genotype-scoring rules were applied as above to obtain consensus genotypes for each individual.

Temporal change in genetic diversity

Marker polymorphism was assessed by determining number of alleles/locus (*Na*), number of unique alleles/locus (*Pa*), observed heterozygosity (H_o), and expected heterozygosity (H_e) for each locus for each population, and across loci for each population, using F-STAT V.2.9.3.2 (Goudet 2002). As sample size can effect estimates of allelic diversity (*Na* and *Pa*), per-locus and overall unbiased estimates of allelic richness (*Ar*) and unique allelic richness (*Pr*), taking into account small sample size, were calculated for each population using rarefaction within HP-RARE V.1.1 (Kalinowski 2004, 2005), applying a minimum sample of seven diploid individuals (i.e. number of genes, *g*=14).

Differences in diversity between current and historical populations, and potential diversity loss over time, were assessed by comparing *Na* and H_e. *Ar* is more sensitive to bottleneck effects than other diversity measures using microsatellite data: alleles can be lost rapidly from these loci via genetic drift following a bottleneck, with allelic diversity (dependent on effective population size and within-population allele number/frequency) declining more rapidly than heterozygosity (dependent only on effective population size) (Spencer et al. 2000, Leberg 2002, Keller et al. 2012). We further compared *Ar* (and *Pr*) between current

and historical populations to determine if declines were due to loss of alleles from the historical population. As samples were limited (<2*g*=28) for both populations, the non-parametric Wilcoxon signed-rank test within R V.3.0.1 (R Development Core Team 2013) was used to investigate declines.

Genetic differentiation of current and historical populations

Differentiation between current and historical populations was examined with the fixation index (pairwise F_{ST}; Cockerham and Weir 1993), calculated using F-STAT V.2.9.3.2 with a randomisation approach to test for significance (Goudet 2002); with Principal Coordinates Analysis (PCoA) using pairwise genetic distances between all samples within GenAlEx V.6.5 (Peakall and Smouse 2006); and with a Bayesian clustering approach within STRUCTURE V.2.3.4 (Pritchard et al. 2000). We assessed extent of partitioning by exploring a range of values for the number of populations prior, '*K*', with *K*=1-8 and five replicates/*K*, using 100,000 iterations following a burn-in of 10,000 iterations, after which we obtained consistent and convergent results (Pritchard et al. 2000, 2010). An admixture model and independent allele frequencies were adopted, as appropriate for closely-related populations but where allele frequencies may be reasonably different (Pritchard et al. 2000). Optimal *K* was determined using the Δ*K* approach (Evanno et al. 2005) implemented in STRUCTURE HARVESTER V.0.6.93 (Earl and vonHoldt 2012). Related individuals within a sample may create a false signal of population genetic structure or overestimate cluster number (Rodríguez-Ramilo and Wang 2012); we confirmed the pattern and extent of population structure using CLUSTER_DIST, which maximizes between-group genetic distances and

274 does not make Hardy-Weinberg and linkage equilibrium assumptions (Rodríguez-Ramilo et
275 al. 2014) (Appendix S5).

276

277 *Population bottleneck*

278 Genetic evidence for a past bottleneck was assessed via graphical investigation of mode allele
279 frequency shift between historical and current populations (Luikart et al. 1998) by grouping
280 alleles across polymorphic loci for each population into 10 frequency classes (0.001-0.100,
281 0.101-0.200 etc, until 1.0) and comparing resultant histograms. We also used
282 BOTTLENECK V.1.2.02 (Cornuet and Luikart 1996) to assess mode-shift and evaluate
283 heterozygosity excess under four mutation models, using three significance tests (sign,
284 standardised differences, Wilcoxon sign-rank). Microsatellites rarely confirm strictly to either
285 the infinite allele mutation (IAM) model or stepwise mutation model (SMM), therefore a two-
286 phase model (TPM) accommodating both mutation types (Di Rienzo et al. 1994, Piry et al.
287 1999) was also adopted with 70% and 90% SMM respectively (thus 30% and 10% IAM).

288

289 *Effective population size*

290 To determine current effective population size (N_e) and assess evidence for temporal change
291 in N_e we employed multiple 'single-sample' approaches: linkage disequilibrium,
292 heterozygosity excess and molecular coancestry within NeEstimator V.2.0 (Do et al. 2013),
293 and full-likelihood sib-ship assignment (Wang 2009) within COLONY V.2.0.4.5 (Jones and
294 Wang 2010). We also adopted a Bayesian approach implemented in TMVP (Beaumont
295 2003), sampling independent genealogical histories from temporally-spaced gene frequency
296 data (all samples, pooled) to give a posterior distribution of estimated historical N_e (time of

297 oldest historical sample) and current N_e (time of youngest sample). Allele frequencies were
298 calculated for dated historical and current samples, with time measured in gibbon generations
299 (15 years; Chivers et al. 2013) since sample collection, and a rectangular (uniform) prior of
300 (0,5000) employed for estimation of historical and current N_e. We determined joint mode of
301 the posterior distribution of historical and current N_e estimates, discarding the first 1% (100
302 estimates) of the simulated 10,000 estimate chain as burn-in, applying a smoothing parameter
303 of α=0.6 (after exploring α=0.3-0.7) within R V.3.0.1 (R Development Core Team 2013).
304 This α-value was subsequently employed to determine the 95% higher posterior density
305 (HPD) limits of each N_e, as it produced a sharp joint mode located away from the upper limit
306 of priors for either N_e.

307

308 *Inbreeding and relatedness*

309 Inbreeding within current and historical populations was assessed by comparing H_e to H_o and
310 assessing the inbreeding coefficient (F_{IS}) (Weir and Cockerham 1984). Inbreeding-driven
311 deviations from HWE were evaluated by estimating F_{IS} for each locus and across all loci for
312 each population using F-STAT V.2.9.3.2 (Goudet 2002). Relationships between individuals
313 in the current population were investigated using COLONY V.2.0.4.5 (Jones and Wang 2010)
314 to infer parentage and full/half sib-ship relations over the entire population, and determine the
315 best configuration of relationships under maximum likelihood (ML). We used a 0.01
316 genotyping error rate, polygamous mating system, and 0.5 probability that an actual
317 father/mother of an offspring was included in candidate father/mother datasets. As all current
318 samples originated from one population, we retained all links from the best configuration.
319 The coefficient of relatedness (r) was determined using ML-RELATE (Kalinowski et al.

2006) to estimate pairwise relatedness between all individuals in the current population. ML *r* estimates and ML configuration indicated degree and structure of relatedness within the current population over >1 generation to reveal probable shared parentages that likely produced observed relatedness/relationships.

Sex ratios

Sex-determination (Amelogenin) consensus genotypes were used to calculate sex ratios of all individuals sampled from the current population, all individuals in Group B, and immature offspring within Group B.

Results

Marker characteristics

No evidence of null alleles, allelic dropout, or scoring error due to stutter was detected for the final 13 loci. LOSITAN simulation results identified two loci (D17S804, D20S206) falling outside the 95% quantile for neutral markers; data for these loci were excluded from further analyses. Significant linkage disequilibrium was detected between two further pairs of loci in the current population and one pair in the historical population ($P<0.05$; Appendix S6); these patterns were not consistent across both populations or the entire sample for any one pair of loci, so all 11 loci were retained given the small final number of markers. Consensus genotypes for these loci were obtained for nine living individuals and eight museum specimens (final dataset: mean quality index=0.78, missing data percentage=2.67%), representing 36% of the current global population and 61.5% of known historical specimens.

342

343 *Temporal change in genetic diversity*

344 Genetic diversity in both temporal 'populations' was low, with small *Na*, *Ar* and H_e values
345 observed for each locus, and small overall average values for each population (current:
346 *Na*=2.273, *Ar*=2.240, H_e=0.431; historical: *Na*=3.364, *Ar*=3.290, H_e=0.626; Table 1). Current
347 diversity was lower than historical diversity for all metrics (Table 1); across all loci, *Na* was
348 32% lower in the current population (one-sided Wilcoxon W=99.5, *P*=0.0031), *Ar* was 32%
349 lower (W=103, *P*=0.0023), and H_e was 31% lower (W=97, *P*=0.0090). 'Historical' alleles
350 have been lost over time, with alleles in the historical population absent from the current
351 population at seven of 11 loci, and a significantly lower current *Pr* (W=93, *P*=0.0139).

352

353 *Genetic differentiation of current and historical populations*

354 Current and historical populations showed significant differentiation, with 16% of total
355 observed genetic variation distributed between populations and 84% within populations
356 (pairwise F_{ST}=0.156, *P*=0.0315), suggesting substantial divergence of the current population.
357 PCoA confirmed temporal differentiation between populations, and revealed additional
358 divergence patterns (Figure 1). Current samples clustered along axis 2, but the eldest living
359 individual (B1) and Groups A and C individuals diverged slightly from other Group B
360 individuals on axis 1. Historical samples dispersed along both axes, clustering loosely but
361 away from current samples, indicating greater genetic variation compared to current samples
362 and divergence between current and historical populations.

363 Bayesian cluster analysis distinguished three genetic populations (peak ΔK at K=3 clusters)
364 (Figure 2). Ninety-five percent of current samples fell into one cluster, indicating

differentiation from historical samples (Figure 3). Historical samples subdivided into two populations (47.2%, 49.2%) corresponding approximately to a split between older samples (1899-1911) and younger samples (1960s-1980s); however, one sample from the 1980s (BWL672) clustered more often with older samples as this sample retained some 'older' alleles. Two current samples (B1, C) occasionally clustered with the population comprising samples from 1899-1911 plus BWL672, again due to these individuals retaining alleles otherwise only present in historical populations. This pattern was supported by CLUSTER_DIST analysis, which identified additional clusters in the historical sample (corresponding to further temporal partitioning of specimens) but grouped most current samples into one cluster/population, and assigned two current samples (B1, C) to another cluster along with two historical samples (Appendix S5), indicating that observed population structure was not solely the result of close relationships between individuals in the current population.

Population bottleneck

The historical population exhibited the L-shaped allele frequency distribution expected for non-bottlenecked populations; many alleles fall into low-frequency classes (0.001-0.2), and few fall into intermediate-frequency (0.201-0.8) or high-frequency (0.801-1.0) classes (Figure 4). The current population showed fewer alleles in low-frequency classes and more in higher-frequency classes. Mode allele frequency across all loci in the historical population was 0.188, but was higher in the current population at 0.5; this mode-shift was confirmed within BOTTLENECK.

For the current population, all three significance tests indicated significant heterozygosity excess under IAM. Two tests indicated significant excess under the more conservative TPM with 70% SMM; TPM with 90% SMM indicated heterozygosity excess using the Wilcoxon test only (Table 2). Significant heterozygosity excess was also revealed in the historical population for two of three tests under IAM and TPM with 70% SMM, and from the Wilcoxon test under TPM with 90% SMM. Although the test statistic only approaches a normal distribution if >20 loci are used (Cornuet and Luikart 1996), the non-parametric Wilcoxon test remains robust using few polymorphic loci (Piry et al. 1999), and indicates both current and historical populations show a genetic signal consistent with a bottleneck.

Effective population size

All single-sample estimates reported exceptionally low current N_e (Table 3). Estimates of historical N_e varied substantially, but generally showed slightly larger values. However, large confidence intervals for all estimates limit comparison between temporal populations. This apparent lack of difference in historical versus current N_e could reflect limitations of single-sample approaches for small n, or stable low N_e over time pre-dating mid-20th century decline. Accuracy of single-sample estimates is likely limited, and may only approximate true N_e.

Bayesian estimation of N_e at the time of oldest and youngest samples (1899-2011) was more informative and indicated temporal change in N_e (Figure 5a). Density of points in the posterior distribution is proportional to the probability density of historical and current N_e; the off-diagonal distribution of points indicates that current N_e is not equal to historical N_e. The exceptionally flat posterior distribution, with points densely concentrated along the x-axis

410 (historical N_e), provides strong evidence of decline in N_e between 1899-2011, and indicates
411 current N_e is very low. The joint mode (and 95% HPD limits) for the marginal from the
412 density estimation is: historical N_e=1162.96 (95% HPD limits=55.64-4129.95), current
413 N_e=2.16 (95% HPD limits=0.98-4.18) (Figure 5b). Estimation of historical N_e is somewhat
414 uncertain, indicated by the large 95% HPD limit range; however, N_e was certainly larger in
415 the late 19th century.

416

417 *Inbreeding and relatedness*

418 For the current population, H_o was generally greater than H_e at each locus (nine of 11 loci),
419 producing an overall trend of mean H_o > mean H_e (Table 1). The opposite was true of the
420 historical population (H_e>H_o for eight of 11 loci). Although no estimated F_{IS} values were
421 significant for any locus or overall for the historical population (all *P*-values>0.05), F_{IS} values
422 of three loci and the overall estimate for current population were significant (all *P*-
423 values<0.05), indicating non-random mating (outbreeding) within the current population.

424 Overall, ML *r* estimates indicated a high level of relatedness between all individuals in the
425 current population (Appendix S7). Average relatedness across sampled individuals was high,
426 roughly between half- and full-sibs (mean r=0.34±0.05). Average relatedness within Group B
427 was approximately full-sibs or parent-offspring (mean r=0.45±0.07). Between social groups,
428 relatedness was slightly lower, between cousins and half-sibs. Relatedness of Groups B and
429 C (mean r=0.18±0.09) was similar to that of Groups A and B (mean r=0.16±0.07),
430 approximately at the level of cousins; relatedness of Groups A and C was slightly higher
431 (r=0.38, no S.E. as comparison between two individuals only), at least half but almost full-
432 sibs. ML configuration of relationships supported these results and revealed additional

between-individual relationships, including some that suggest possible past inbreeding (Appendix S7).

Sex ratios

Amelogenin consensus genotypes were obtained for all sampled individuals from the current population, and confirmed the sex in all cases where an individual's sex was already known (three males, two females). The overall sampled population's sex ratio was two males per female (Table 4). Group B had a ratio of four males to three females; this group's immature offspring included three males and one female.

Discussion

This study represents the first investigation of the genetic status of the sole remaining Hainan gibbon population within the context of its genetic history, and demonstrates this population is genetically impoverished following a substantial crash in N_e consistent with the species' range decline. Both historical and current samples show low levels of polymorphism compared with limited available data on diversity in other gibbon populations (*Hylobates lar*: *Na*=7.0, H_e=0.725, Chambers et al. 2004; *H. muelleri*: *Na*=14.8, Oka and Takenaka 2001; Appendix S3). Although this low diversity may partly reflect cross-amplification, these other studies also cross-amplified many of the same human microsatellites and found greater diversity. Furthermore, despite low diversity in both samples, we detected significant reductions in heterozygosity and allelic diversity over time, indicating that low diversity is not a long-term pattern; instead, diversity has declined since the 19th century, and even further within recent decades. This decline was detected even with limitations to available data

456 (sample size, number of loci amplified across all samples); these estimates are therefore
457 probably conservative with respect to amount of diversity lost. This detected decline also
458 corresponds with differences in diversity reported for many threatened species, which on
459 average possess c.65% of microsatellite diversity of phylogenetically related, non-threatened
460 taxa (Frankham et al. 2009). Similar diversity declines following severe bottlenecks are
461 known from other threatened species: H_e declined by 52% and *Na* by 32% in black-footed
462 ferrets (*Mustela nigripes*) (Wisely et al. 2002); and H_e declined by 57% and *Na* by 55% in
463 Mauritius kestrels (*Falco punctatus*) (Groombridge et al. 2000). 'Ghost' alleles (Bouzat et al.
464 1998) present in the historical gibbon population were absent from the current population for
465 several loci, indicating loss of alleles over time due to a bottleneck. The overall pattern
466 detected by PCoA, with broadly distributed historical samples and tightly clustered current
467 samples, also supports the detected diversity decline, likely reflecting 20th century gibbon
468 range contraction across Hainan (Appendix S1; Liu et al. 1984, Zhou et al. 2005).

469 This decline has led to significant differentiation of current and historical populations.
470 Observed differentiation is virtually identical to differentiation in island populations of Kloss'
471 gibbon (*Hylobates klossii*) that have been isolated for c.7,000 years (F_{ST}=0.157; Whittaker
472 2009), and comparable to that in partially/completely disconnected subpopulations of other
473 threatened mammals, e.g. Sumatran tiger (*Panthera tigris sumatrae*) (Smith 2012), Ethiopian
474 wolf (*Canis simensis*) (Gottelli et al. 2004). Assignment of 5% of the current population to
475 inferred historical populations (Figure 3) indicates that most current-day individuals have a
476 different genetic signature to the historical population, but a small amount of historical
477 genetic structure may persist today, contained within genotypes of two sampled individuals
478 (B1, C). As B1 is already post-reproductive, having not given birth since 2000 (based on

479 ongoing field observations: Li et al. 2010, Bryant 2014), diversity will decline further
480 following her death and current population structure may become increasingly distinct.

481 Island species may show diminished genetic diversity resulting from founder effects,
482 evolutionary histories of sustained isolation and small N_e, or recent population crashes
483 (Frankham 1997, Groombridge et al. 2009). Evidence of a recent bottleneck having produced
484 the observed diversity reduction in the current gibbon population is compelling. There has
485 been a clear shift in mode of allele frequencies between temporal populations, and clear
486 reduction in N_e from time of the oldest sample. The observed ratio of current N_e to current
487 population size (2.16:25=0.086) compares closely to that found across 102 species (mean
488 ratio=c.0.10; Frankham 1995), providing support for our N_e estimate. The acutely low N_e is
489 particularly alarming, as previous studies indicate that N_e<50 can significantly decrease
490 population viability (Westemeier et al. 1998, Madsen et al. 1999), with N_e≥1,000 required for
491 long-term evolutionary potential (Frankham et al. 2014). Our analysis does not permit
492 determination of exact timing of population crash, but the steepest decline probably occurred
493 after collection of the youngest historical samples (1980), when Hainan experienced extensive
494 deforestation (Li 2004) and BNNR received limited financial support due to changing
495 political administration in the early 1990s, reportedly leading to forest loss and gibbon
496 poaching (Zhang 1992, Zhang and Sheeran 1993).

497 Interestingly, there is also evidence for an older bottleneck, with significant heterozygosity
498 excess and small N_e (estimated by single-sample metrics) detected for both temporal samples,
499 and relatively low polymorphism already shown by historical samples. This could reflect
500 limitations of approaches employed for given sample sizes: all single-sample N_e estimators
501 lose power with small sample sizes (n<20 individuals) and/or restricted numbers of markers

(England et al. 2006, Wang 2009). Indeed, single-sample estimates of historical N_e (2.8-16) were lower than expected for the historical period (1899-1980); anecdotal population estimates suggest c.2,000 individuals remained in the 1950s (Liu et al. 1984), and c.500 individuals in the 1970s (Zhou et al. 2005). Other factors beyond bottlenecks can generate heterozygote excess, especially in small populations (e.g. selection, dieocy, unequal sex ratios, polygamous mating; Storz et al. 2001, Balloux 2004), potentially explaining this pattern. However, Bayesian assessment of changing N_e over time revealed both temporal decline in N_e, supporting past population bottleneck, and a more realistic historical N_e, indicating that population size when the oldest sample was collected was already relatively small; the N_e of c.1,000 implies a population of c.10,000 gibbons in the late 19th century, although the large HPD confidence limits show uncertainty around this estimation. Possible reduced Hainan-wide gibbon abundance >100 years ago is supported by contemporary historical accounts, which describe the species as already rare (Swinhoe 1870). Other gibbon species are also known to have declined across China by the 19th century (Wen 2009), and other mammals reportedly present in Hainan during the Ming-Qing dynasties (e.g. records possibly referring to dhole *Cuon alpinus* and Père David's deer *Elaphurus davidianus*) had disappeared by the 20th century (Dobroruka 1970, Wen 2009) likely due to historical persecution or overexploitation, suggesting that Hainan's mammal fauna was already being impacted by human activities. The Hainan gibbon may therefore have suffered substantial decline even before its 20th century population crash.

We found evidence for a reduction in inbreeding due to non-random mating in the current population, with heterozygote excess (F_{IS}<0), indicating that the species' mating behaviour may favour mating between less related individuals. Polygynous mating can generate heterozygote excess in populations characterised by such mating, which can produce negative

526 F_{IS} values (Storz et al. 2001). The Hainan gibbon forms large polygynous groups which may
527 be the normal social structure for this species (Bryant et al. 2015); this and/or other factors
528 which create unequal sex-specific gene frequencies through binomial sampling error may
529 have driven observed heterozygote excess (Balloux 2004). Inbreeding is lower than expected
530 under random mating, but must still be high given evidence for mating between related
531 individuals in the population pedigree. This is unsurprising if we consider the 'pedigree'
532 definition of inbreeding (individuals are considered inbred when parents are related) instead
533 of the 'non-random mating' (F_{IS}) definition (Keller and Waller 2002), as mating between
534 relatives will occur in small populations even under random mating (Keller et al. 2012).
535 Indeed, individuals in the current population appear related at the level of half- to full-siblings
536 between social groups, and full-siblings or parent-offspring within Group B. Crosses at even
537 half-sibling level will theoretically increase non-random mating and the inbreeding coefficient
538 by 0.15 after only two generations, reaching 1.0 (complete inbreeding) after c.20 generations
539 (Hartl and Clark 1997). Li et al. (2010), using only six samples from one group, reported four
540 haplotypes within Group B. Our results indicate higher levels of relatedness, with only two
541 maternal lines present in this group's pedigree (Appendix S6). These results may reflect
542 restricted sampling and/or low polymorphism for the few loci genotyped, implying closer
543 relationships than actually exist (Kalinowski et al. 2006, Jones and Wang 2010). However,
544 our pedigree was derived from more extensive sampling, and is likely to represent relatedness
545 more accurately than the previous assessment. Kenyon et al. (2011) detected full-sibling
546 relationships between adults from neighbouring yellow-cheeked gibbon (*Nomascus*
547 *gabriellae*) groups in Vietnam, suggesting levels of relatedness between Hainan gibbon social
548 groups seem realistic; while direct comparisons are limited by differences in methodology and
549 social/mating systems, studies of other bottlenecked populations of threatened taxa have

revealed similarly elevated relatedness levels within social clusters (Taylor et al. 1997, Hagell et al. 2013). Data limitations for other gibbons make it difficult to assess whether our results indicate closer-than-average relatedness; however, as there are no unrelated potential mates within the remaining Hainan gibbon population, mating between individuals with high levels of relatedness and thus inbreeding, along with probability of genetic identity by descent, are already very high and will only increase.

Molecular sex determination suggested a male-biased offspring ratio in Group B, supporting previous suggestions based upon visual observations (Liu et al. 1989). Assuming equal probability to produce either sex, we might observe a ratio at least this male-biased 7.3% of the time by chance. However, several small *ex situ* gibbon populations display similarly male-skewed sex ratios at birth; Jago and Melfi (2010) detected male bias of 67-90% for three gibbon species kept in zoos despite captive management, and demonstrated a significant statistical association with gross energy within captive diet, with females on lower-calorie diets more likely to produce male offspring. Given concerns regarding potentially suboptimal habitat quality at Bawangling, which may be close to gibbon elevational limits and ecologically marginal (Chan et al. 2005, Turvey et al. 2015), these findings have important management implications.

Our study possesses limitations inherent in all conservation genetics studies of extremely rare species: issues of sample size and potentially reduced statistical power, with inferential power of diversity analyses and detection of diversity declines constrained by small *n,* unavoidably biased sampling of the current population, and number of loci used to characterise past and present genetic status. However, *n* has been found to have little effect on H_e or pairwise F_{ST}, even with only five individuals genotyped at 10 loci (Smith 2012). Furthermore, we detected

573 significant biological effects despite our reduced sample, meaning that these effects must be
574 substantial to be detected. Although low diversity detected within the current population may
575 partly reflect sampling bias (seven individuals from one group, but only one individual each
576 from other groups), detection of a bottleneck during the last century suggests that observed
577 diversity more likely reflects drastic population reduction, and our results are consistent with
578 historical population size estimates (Liu et al. 1984, 1989, Zhou et al. 2005).

579 Detected diversity declines may reflect temporal and spatial sampling bias, both unavoidable
580 constraints of limited sample availability. Such drawbacks to temporal comparisons in
581 critically small populations are not unique to this study (Groombridge et al. 2000, 2009,
582 Gottelli et al. 2004, Holbrook et al. 2012). Historical samples with successful DNA
583 extraction (n=8) spanned an 81-year period and were probably collected from localities across
584 Hainan (Appendix S2). By comparison, current samples represent a temporal snapshot from
585 one location. However, at least half the successfully amplified historical samples were
586 definitely from the same location as current samples (Bawangling region), reducing spatial
587 sampling bias; remaining samples lack adequate collection data to determine precise
588 geographic provenance. Bayesian assessment of change in N_e, incorporating dates for each
589 sample, supported a past population bottleneck. There was also clear evidence of genetic
590 differentiation between current and historical samples, indicating a shift in genetic
591 composition of the species over time. Additionally, even in the absence of historical context,
592 remaining diversity in the current population is exceptionally low. This is unlikely to
593 represent genotyping error, as conservative genotyping rules were employed to derive
594 consensus genotypes. The population also shows other hallmarks of being genetically
595 compromised (e.g. high level of relatedness).

596 Reduced genetic diversity and extremely low N_e in the current population may have important
597 implications for long-term viability, potentially increasing vulnerability to disease, and
598 hampering its ability to respond to sudden environmental variation and potential future
599 climate change effects at Bawangling (Lindsey et al. 2013). Highly threatened species can
600 sometimes persist for long periods despite reduced genetic diversity (e.g. Iberian lynx *Lynx*
601 *pardinus* (≥50,000 years), Rodríguez et al. 2011; koala *Phascolarctos cinereus* (≥120 years),
602 Tsangaras et al. 2012); however, such species have typically consisted of >1 population,
603 making them less vulnerable to stochastic effects that could eliminate the last Hainan gibbon
604 population. A strategy of 'genetic rescue', where genes are introduced from other wild or
605 captive populations to improve the genetic state of a population with low genetic diversity
606 (Hedrick and Fredrickson 2010), is also not an option, as the Bawangling population
607 constitutes the only known population. Thankfully, other species have managed to recover
608 from critically low sizes without addition of new genetic variation, despite severe losses of
609 genetic variability following extreme bottlenecks, e.g. Chatham Islands black robin (*Petroica*
610 *traversi*) (Ardern and Lambert 1997), Mauritius kestrel (Groombridge et al. 2000), Mauritius
611 parakeet (*Psittacula echo*) (Raisin et al. 2012). Such recoveries have only been achieved
612 through intensive, carefully planned management, indicating reduced genetic diversity may
613 not preclude conservation success, but must be considered during conservation planning.

614 Long-term Hainan gibbon recovery will likely require intensive management, for example
615 potential translocation of individuals to establish new founder populations, and our findings
616 have important implications in this regard. As all sampled individuals are related at the level
617 of half- to full-siblings, it is essential to consider data on relatedness when deciding potential
618 management actions, although attempts to maintain genetic integrity must be coupled with
619 maintenance of social integrity for gregarious, group-living species with complex social

620 behaviours such as gibbons. The close observed relationships and evidence of inbreeding
621 indicate it may be necessary to adjust potential expectations of likely population recovery
622 rates, as lowered reproductive fitness and reduced survival are known in other populations
623 experiencing inbreeding (Swinnerton et al. 2004, Hemmings et al. 2012), and a crucial next
624 step is to incorporate our data on Hainan gibbon genetic diversity and relatedness into
625 population viability analysis (Turvey et al. 2015). Attention should focus on preservation of
626 all remaining gibbon individuals to prevent further diversity declines and losses to the
627 breeding pool. Consequently, eliminating the threat of hunting is absolutely paramount, as is
628 reduction of other anthropogenic activities currently degrading habitat at Bawangling (illegal
629 forest clearance, non-timber forest product collection, livestock grazing, infrastructure
630 development for tourism; Zhang et al. 2010, Turvey et al. 2015). Increasing available habitat
631 may also support population growth, reduce environmental impacts to the offspring sex-ratio,
632 and allow the population to withstand localised environmental threats in the face of its
633 reduced diversity.

634 Our study demonstrates that despite small sample sizes and challenges to data collection, it is
635 possible to generate comprehensive new baseline datasets regarding the genetic status of
636 Critically Endangered species through use of multiple analytical techniques, with resultant
637 information providing crucial insights to inform conservation management. Our research also
638 adds to a growing body of literature (Groombridge et al. 2009, Bristol et al. 2013, Tollington
639 et al. 2013) demonstrating the importance of contextualising measures of genetic condition
640 for threatened populations against their historical genetic status and chronologies of past
641 population dynamics and human impacts, to reveal nuanced insights required for conservation
642 management. Moving forward, further assessment of the robustness of existing statistical
643 methods to tiny samples sizes, and development of new metrics or analytical frameworks to

644 accommodate these issues, will be crucial to facilitate genetic studies on species of extreme
645 rarity.

646

647 **Acknowledgements**

648 This research was made possible through collaboration with Bawangling National Nature
649 Reserve Management Office, Hainan Bawangling Forestry Bureau, and Hainan Provincial
650 Forestry Department. We thank the Hainan Gibbon Monitoring Team, Wang Chungdong,
651 Selina Brace, Ian Barnes, Jim Groombridge, Linda Vigilant, Benoit Goossens, Guy
652 Cowlishaw, Jinliang Wang and Jing Chai. We are also grateful to the editor and three
653 anonymous reviewers for useful comments which helped improve and clarify our manuscript.
654 Historical samples were provided by: Natural History Museum, London; National Museum of
655 Ireland, Dublin; Museum für Naturkunde, Berlin; South China Institute of Endangered
656 Animals, Guangzhou. Funding was provided by: Ocean Park Conservation Foundation, Hong
657 Kong; Mohamed bin Zayed Species Conservation Fund; Daisy Balogh Memorial Travel
658 Fund; Harold and Olga Fox Fund; UCL Overseas Research Scholarship; IRTSTYN Yunnan
659 University; Royal Society University Research Fellowship (UF080320).

660

661 **References**

662 Antao T, Lopes A, Lopes R *et al*. (2008) LOSITAN: A workbench to detect molecular
663 adaptation based on a F_{ST}-outlier method. *BMC Bioinformatics*, **9**, 323.

664 Ardern SL, Lambert DM (1997) Is the black robin in genetic peril? *Molecular Ecology*, **6**, 21-
665 28.

666 Balloux F (2004) Heterozygote excess in small populations and the heterozygote-excess
667 effective population size. *Evolution*, **58**, 1891-1900.

668 Beaumont MA (2003) Estimation of population growth or decline in genetically monitored
669 populations. *Genetics*, **164**, 1139-1160.

670 Bouzat JL, Lewin HA, Paige KN (1998) The ghost of genetic diversity past: historical DNA
671 analysis of the greater prairie chicken. *The American Naturalist*, **152**, 1-6.

672 Bradley BJ, Chambers KE, Vigilant L (2001) Accurate DNA-based sex identification of apes
673 using non-invasive samples. *Conservation Genetics*, **2**, 179-181.

674 Bristol RM, Tucker R, Dawson DA *et al*. (2013) Comparison of historical bottleneck effects
675 and genetic consequences of re-introduction in a Critically Endangered island passerine.
676 *Molecular Ecology*, **22**, 4644-4662.

677 Bryant JV, Olson VA, Chatterjee HJ *et al.* (2015) Identifying environmental versus
678 phylogenetic correlates of behavioural ecology in gibbons: implications for conservation
679 management of the world's rarest ape. *BMC Evolutionary Biology*, **15**, 171-183.

680 Chambers KE, Reichard UH, Möller A *et al*. (2004) Cross-species amplification of human
681 microsatellite markers using noninvasive samples from white-handed gibbons (*Hylobates*
682 *lar*). *American Journal of Primatology*, **64**, 19-27.

683 Chan BPL, Fellowes JR, Geissmann T *et al*. (2005) *Hainan Gibbon Status Survey and*
684 *Conservation Action Plan*. Kadoorie Farm and Botanic Garden, Hong Kong.

685 Chan Y, Roos C., Inoue-Murayama M *et al*. (2013) Inferring the evolutionary histories of
686 divergences in *Hylobates* and *Nomascus* gibbons through multilocus sequence data. *BMC*
687 *Evolutionary Biology*, **13**, 82.

688 Chivers DJ, Anandam MV, Groves CP *et al.* (2013) Family Hylobatidae (gibbons). In:
689 *Handbook of the Mammals of the World*, Volume 3: Primates (eds Mittermeier RA, Rylands
690 AB, Wilson DE), pp. 754-791. Lynx Edicions, Barcelona.

691 Clisson I, Lathuilliere M, Crouau-Roy B (2000) Conservation and evolution of microsatellite
692 loci in primate taxa. *American Journal of Primatology*, **50,** 205-214.

693 Cockerham CC, Weir BS (1993) Estimation of gene flow from F-statistics. *Evolution*, **47**,
694 855-863.

695 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting
696 recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001-2014.

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

699 Di Rienzo AA, Peterson C, Garza JC *et al.* (1994) Mutational processes of simple-sequence
700 repeat loci in human populations. *Proceedings of the National Academy of Sciences USA*, **91**,
701 3166-3170.

702 Do C, Waples RS, Peel D *et al*. (2013) NeEstimator v2.0: re-implementation of software for
703 the estimation of contemporary effective population size (N_e) from genetic data. *Molecular*
704 *Ecology Resources*, **14**, 209-214.

Dobroruka LJ (1970) To the supposed formerly occurrence of the David's deer *Elaphurus davidianus* Milne-Edwards, 1866, in Hainan. *Mammalia*, **34**, 162-164.

Earl D, vonHoldt B (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359-361.

England PR, Cornuet JM, Berthier P *et al.* (2006) Estimating effective population size from linkage disequilibrium: severe bias in small samples. *Conservation Genetics*, **7**, 303-308.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Fellowes JR, Chan BPL, Zhou J *et al*. (2008) Current status of the Hainan gibbon (*Nomascus hainanus*): progress of population monitoring and other priority actions. *Asian Primates Journal*, **1**, 2-9.

Fischer A, Pollack J, Thalmann O *et al.* (2006) Demographic history and genetic differentiation in apes. *Current Biology*, **16**, 1133-1138.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genetics Research*, **66**, 95-107.

Frankham R (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, **78**, 311-327.

Frankham R, Ballou JD, Briscoe DA (2009) *An Introduction to Conservation Genetics*, 2nd edition. Cambridge University Press, Cambridge, 644 pp.

Frankham R, Bradshaw CJA, Brook BW (2014) Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, **170**, 56-63.

Goossens B, Anthony N, Jeffery K *et al.* (2003) Collection, storage and analysis of non-invasive genetic material in primate biology. In: *Field and Laboratory Methods in Primatology: A Practical Guide* (eds Setchell JM, Curtis DJ), pp. 295-308. Cambridge University Press, Cambridge.

Goossens B, Chikhi L, Jalil MF *et al.* (2005) Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. *Molecular Ecology*, **14**, 441-456.

Goossens B, Chikhi L, Utami S *et al.* (2000a) A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. *Conservation Genetics*, **1**, 157-162.

Goossens B, Latour S, Vidal C *et al.* (2000b) Twenty new microsatellite loci for use with hair and faecal samples in the chimpanzee (*Pan troglodytes troglodytes*). *Folia Primatologica*, **71**, 177-180.

741 Gottelli D, Marino J, Sillero-Zubiri C *et al.* (2004). The effect of the last glacial age on
742 speciation and population genetic structure of the endangered Ethiopian wolf (*Canis*
743 *simensis*). *Molecular Ecology*, **13**, 2275-2286.

744 Goudet J (2002) *FSTAT (Version 2.9.3.2), computer program for PCs*. Institute of Ecology,
745 Lausane, Switzerland.

746 Grantham HS, Wilson KA, Moilanen A *et al.* (2009) Delaying conservation actions for
747 improved knowledge: how long should we wait? *Ecology Letters*, **12**, 293-301.

748 Groombridge JJ, Dawson DA, Burke T *et al.* (2009) Evaluating the demographic history of
749 the Seychelles kestrel (*Falco araea*): genetic evidence for recovery from a population
750 bottleneck following minimal conservation management. *Biological Conservation*, **142**, 2250-
751 2257.

752 Groombridge JJ, Massey JG, Bruch JC *et al.* (2004) An attempt to recover the po'ouli by
753 translocation and an appraisal of recovery strategy for bird species of extreme rarity.
754 *Biological Conservation*, **118**, 365-375.

755 Groombridge JJ, Jones CG, Bruford MW *et al.* (2000) Ghost alleles of the Mauritius kestrel.
756 *Nature*, **403**, 616-616.

757 Hagell S, Whipple AV, Chambers CL (2013) Population genetic patterns among social groups
758 of the endangered Central American spider monkey (*Ateles geoffroyi*) in a human-dominated
759 landscape. *Ecology and Evolution*, **3**, 1388-1399.

760 Hartl DL, Clark AG (1997) *Principles of Population Genetics*, 4th edition. Sinauer
761 Associates, Sunderland, Massachusetts, 565 pp.

762 Hedrick PW, Fredrickson R (2010) Genetic rescue guidelines with examples from Mexican
763 wolves and Florida panthers. *Conservation Genetics*, **11**, 615-626.

764 Hemmings NL, Slate J, Birkhead TR (2012) Inbreeding causes early death in a passerine bird.
765 *Nature Communications*, **3,** 863.

766 Hoffmann M, Hilton-Taylor C, Angulo A *et al*. (2010) The impact of conservation on the
767 status of the world's vertebrates. *Science*, **330**, 1503-1509.

768 Holbrook JD, DeYoung RW, Tewes ME *et al.* (2012) Demographic history of an elusive
769 carnivore: using museums to inform management. *Evolutionary Applications*, **5**, 619-628.

770 Jago N, Melfi VA (2010) Sex ratios in captive gibbons. *Zoo Research News*, **11**, 2-3.

771 Johnson WE, Onorato DP, Roelke ME *et al.* (2010) Genetic restoration of the florida panther.
772 *Science*, **329**, 1641-1645.

773 Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from
774 multilocus genotype data. *Molecular Ecology Resources*, **10**, 551-555.

775 Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical
776 sampling designs. *Conservation Genetics*, **5**, 539-543.

777 Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on
778 measures of allelic richness. *Molecular Ecology Notes*, **5**, 187-189.

779 Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for
780 maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**,
781 576-579.

782 Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and*
783 *Evolution*, **17**, 230-41.

784 Keller LF, Biebach I, Ewing SR *et al.* (2012) The genetics of reintroductions: inbreeding and
785 genetic drift. In: *Reintroduction Biology: Integrating Science and Management* (eds Ewan JG,
786 Armstrong DP, Parker KA *et al.*), pp. 360-394. Wiley-Blackwell, Oxford.

787 Kenyon M, Roos C, Binh VT *et al.* (2011) Extrapair paternity in golden-cheeked gibbons
788 (*Nomascus gabriellae*) in the secondary lowland forest of Cat Tien National Park, Vietnam.
789 *Folia Primatologica*, **82**, 154-164.

790 Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks.
791 *Molecular Ecology*, **11**, 2445-2449.

792 Li Y (2004) Past, present and future of Hainan tropical rainforests. *Living Forests*, **7**, 9-12.

793 Li Z, Wei F, Zhou J (2010) Mitochondrial DNA D-loop sequence analysis and population
794 rejuvenation of Hainan gibbons (*Nomascus hainanus*). *Biodiversity Science*, **18**, 523–552.

795 Lindsey HA, Gallie J, Taylor S *et al.* (2013) Evolutionary rescue from extinction is contingent
796 on a lower rate of environmental change. *Nature*, **494**, 463-467.

797 Liu Z, Tan C (1990) An analysis on habitat structure of the Hainan gibbon. *Acta Theriologica*
798 *Sinica*, **10(3)**, 163-169.

799 Liu Z, Zhang Y, Jiang H *et al.* (1989) Population structure of *Hylobates concolor* in
800 Bawanglin Nature Reserve, Hainan, China. *American Journal of Primatology*, **19**, 247-254.

801 Liu Z, Yu S, Yuan X (1984) Resources of the Hainan black gibbon and its present situation.
802 *Chinese Wildlife*, **6**, 1-4.

803 Luikart G, Allendorf FW, Cornuet JM *et al.* (1998) Distortion of allele frequency distributions
804 provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238-247.

805 Madsen T, Shine R, Olsson M *et al.* (1999) Conservation biology: restoration of an inbred
806 adder population. *Nature*, **402**, 34-35.

807 Miquel C, Bellemain E, Poillot C *et al.* (2006) Quality indexes to assess the reliability of
808 genotypes in studies using noninvasive sampling and multiple□tube approach. *Molecular*
809 *Ecology Notes*, **6**, 985-988.

810 Oka T, Takenaka O (2001) Wild gibbons' parentage tested by non-invasive DNA sampling
811 and PCR-amplified polymorphic microsatellites. *Primates*, **42**, 67-73.

812 Peakall R, Smouse PE (2006) GenAlEx 6. Genetic analysis in Excel: population genetic
813 software for teaching and research. *Molecular Ecology Notes*, **6**, 288-295.

814 Piry S, Luikart G, Cornuet JM (1999) Computer note. BOTTLENECK: a computer program
815 for detecting recent reductions in the effective size using allele frequency data. *Journal of*
816 *Heredity*, **90**, 502-503.

817 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using
818 multilocus genotype data. *Genetics*, **155**, 945-959.

819 Pritchard JK, Wen X, Falush D (2010) *Documentation for Structure Software: Version 2.3*.
820 University of Chicago, Chicago.

821 R Development Core Team (2013). *R: A Language and Environment for Statistical*
822 *Computing. (V.3.0.1).* R Foundation for Statistical Computing, Vienna, Austria.

823 Raisin C, Frantz AC, Kundu S *et al.* (2012) Genetic consequences of intensive conservation
824 management for the Mauritius parakeet. *Conservation Genetics*, **13**, 707-715.

825 Rodríguez R, Ramírez O Valdiosera CE *et al.* (2011) 50,000 years of genetic uniformity in
826 the Critically Endangered Iberian lynx. *Molecular Ecology*, **20**, 3785–3795.

827 Rodríguez-Ramilo ST, Wang J (2012) The effect of close relatives on unsupervised Bayesian
828 clustering algorithms in population genetic structure analysis. *Molecular Ecology Resources*,
829 **12**, 873-884.

830 Rodriguez-Ramilo ST, Toro MA, Wang J *et al.* (2014) Improving the inference of population
831 genetic structure in the presence of related individuals. *Genetics Research*, **96**, e003.

832 Roeder AD, Bonhomme M, Heijmans C *et al.* (2009) A large panel of microsatellite markers
833 for genetic studies in the Infra-Order Catarrhini. *Folia Primatologica*, **80**, 63-69.

834 Segan DB, Bottrill MC, Baxter PWJ *et al.* (2011) Using conservation evidence to guide
835 management. *Conservation Biology*, **25**, 200-202.

836 Siddle HV, Kreiss A, Eldridge MDB *et al.* (2007) Transmission of a fatal clonal tumor by
837 biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial.
838 *Proceedings of the National Academy of Sciences USA*, **104**, 16221-16226.

839 Smith O (2012) *Population genetics and structure of the Sumatran tiger*. PhD Thesis.
840 Imperial College London, London, U.K.

841 Spencer CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of
842 microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology*, **9**, 1517-1528.

843 Storz JF, Bhat HR, Kunz TH (2001) Genetic consequences of polygyny and social structure in
844 an Indian fruit bat, *Cynopterus sphinx*. I. Inbreeding, outbreeding, and population subdivision.
845 *Evolution*, **55**, 1215-1223.

846 Su B, Monda K, Wang W *et al.* (1995) Molecular phylogeny of Chinese concolor gibbons
847 (subgenus *Nomascus*) using noninvasive DNA genotyping. In: *Primate Research and*
848 *Conservation* (eds Xia W, Zhang Y), pp. 55-63. China Forestry Publishing House, Beijing.

Sullivan KM, Mannucci A, Kimpton CP *et al.* (1993) A rapid and quantitative DNA sex test-fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques*, **15**, 636-641.

Sutherland WJ, Pullin AS, Dolman PM *et al.* (2004) The need for evidence-based conservation. *Trends in Ecology and Evolution*, **19**, 305-308.

Swinhoe R (1870) On the mammals of Hainan. *Proceedings of the Zoological Society of London*, **38**, 224-239.

Swinnerton KJ, Groombridge JJ, Jones CG *et al.* (2004) Inbreeding depression and founder diversity among captive and free-living populations of the endangered pink pigeon *Columba mayeri*. *Animal Conservation*, **7**, 353-364.

Taberlet P, Griffin S, Goossens B *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **24**, 3189-3194.

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution*, **14**, 323-327.

Taylor AC, Horsup A, Johnson CN *et al.* (1997) Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **6**, 9-19.

Thinh VN, Mootnick A, Geissmann T *et al.* (2010a) Mitochondrial evidence for multiple radiations in the evolutionary history of small apes. *BMC Evolutionary Biology*, **10**, 74.

Thinh VN, Rawson B, Hallam C *et al.* (2010b) Phylogeny and distribution of crested gibbons (genus *Nomascus*) based on mitochondrial cytochrome b gene sequence data. *American Journal of Primatology*, **72**, 1047-1054.

Tollington S, Jones CG, Greenwood A *et al.* (2013) Long-term, fine-scale temporal patterns of genetic diversity in the restored Mauritius parakeet reveal genetic impacts of management and associated demographic effects on reintroduction programmes. *Biologcial Conservation*, **161**, 28-38.

Tsangaras K, Ávila-Arcos MC, Ishida Y *et al.* (2012) Historically low mitochondrial DNA diversity in koalas (*Phascolarctos cinereus*). *BMC Genetics*, **13**, 92.

Turvey ST (2008) *Witness to Extinction: How We Failed to Save the Yangtze River Dolphin*. Oxford University Press, Oxford, 234 pp.

Turvey ST, Traylor-Holzer K, Bryant JV *et al.* (2015). *International Conservation Planning Workshop for the Hainan Gibbon: Final Report*. Zoological Society of London, London / IUCN SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota, (English and Chinese).

van Oosterhout C, Hutchinson WF, Wills DP *et al.* (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.

Vigilant L, Bradley BJ (2004) Genetic variation in gorillas. *American Journal of Primatology*, **64**, 161-172.

Vilà C, Sundqvist AK, Flagstad Ø *et al.* (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of London B*, **270**, 91-97.

Wang J (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology*, **18**, 2148-2164.

Wang S, Quan G (1986) Primate status and conservation in China. In: *Primates: The Road to Self-Sustaining Populations* (ed Benirschke K), pp. 213-220. Springer-Verlag, New York.

Wasser SK, Houston CS, Koehler GM *et al.* (1997) Techniques for application of faecal DNA methods to field studies of ursids. *Molecular Ecology*, **6**, 1091-1097.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.

Wen R (2009) *The Distributions and Changes of Rare Wild Animals in China*. Chongqing Science and Technology Press, Chongqing, 782 pp.

Westemeier RL, Brawn JD, Simpson SA *et al.* (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695-1698.

Whittaker DJ (2009) Phylogeography of Kloss's gibbon (*Hylobates klossii*) populations and implications for conservation planning in the Mentawai Islands. In: *The Gibbons: New Perspectives on Small Ape Socioecology and Population Biology* (eds Lappan S, Whittaker DJ), pp. 73-89. Springer-Verlag, New York.

Wisely SM, Buskirk SW, Fleming MA *et al.* (2002) Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. *Journal of Heredity*, **93**, 231-237.

Wu W, Wang X, Claro F *et al.* (2004) The current status of the Hainan black-crested gibbon *Nomascus* sp. cf. *nasutus hainanus* in Bawangling National Nature Reserve, Hainan, China. *Oryx*, **38**, 452-456.

Zhang M, Fellowes JR, Jiang X *et al.* (2010) Degradation of tropical forest in Hainan, China, 1991-2008: conservation implications for Hainan Gibbon (*Nomascus hainanus*). *Biological Conservation*, **143**, 1397-1404.

Zhang Y (1995) A molecular phylogeny of gibbons based on DNA sequences. In: *Primate Research and Conservation* (eds Xia W, Zhang Y), pp 50-54. China Forestry Publishing House, Beijing.

Zhang Y (1992) Hainan gibbon (*Hylobates concolor hainanus*) threatened. *Asian Primates*, **2**, 6.

Zhang Y, Sheeran L (1993) Current status of the Hainan black gibbon (*Hylobates concolor hainanus*). *Asian Primates*, **3**, 3.

Zhou J, Wei F, Li M *et al.* (2005) Hainan black-crested gibbon is headed for extinction. *International Journal of Primatology*, **26**, 453-465.

Data Accessibility

A file containing information on individuals genotyped (sample years, locations, types, number of samples and DNA extractions/individual, final locus genotypes, and final multiplex mixes) is available from Dryad Digital Repository: doi:10.5061/dryad.r1mc3. All analyses are described in sufficient detail to enable replication with data provided.

Author Contributions

JVB designed the research, conducted field research, collected, analysed and interpreted the data, and wrote the manuscript. STT conceived and supervised the research and contributed to the manuscript. DG and CD provided laboratory and analytical guidance. ZY and LJ contributed laboratory space and reagents in China. TG provided key specimen samples. ZX, HX, BPLC and JRF supported field research. HJC supervised the research.

Table Captions

Table 1. Genetic diversity for eleven selected polymorphic microsatellite loci, and summary statistics (overall mean or total ±S.E.) of genetic diversity of current and historical populations across all loci. Diversity measures: number of alleles/locus (*Na*); number of unique alleles/locus (*Pa*); allelic richness (*Ar*); unique allelic richness (*Pr*); observed heterozygosity (H_o); expected heterozygosity (H_e). Inbreeding estimator (F_{IS}) was used to detect deviations from HWE for each locus and each population. Overall values (bold) represent population values: average *Na*, *Pa*, *Ar*, *Pr*, H_o, H_e, overall F_{IS}.

Table 2. Results from heterozygosity excess tests in BOTTLENECK using three significance tests and four models of allele mutation (IAM, SMM, two TPM variants). Significant *P*-values (<0.05) indicated in bold.

Table 3. N_e estimates of current and historical populations inferred via linkage disequilibrium (LD), heterozygosity excess (HE), molecular coancestry (Coan.), and full-likelihood sib-ship assignment (FL); 95% confidence intervals in parentheses.

Table 4. Sex ratios for current population and results of tests for deviation from 1:1 sex ratio using Yates-corrected Pearson's Chi-squared statistic. Degrees of freedom for all tests=1; sample size used to calculate ratios indicated.

959 **Figure Captions**

960

961 **Figure 1.** PCoA based on pairwise genetic distances between historical (squares) and current
962 (diamonds) samples. Proportion of total variance explained by each axis indicated in
963 parentheses; dashed oval encircles current samples.

964

965 **Figure 2.** Second-order rate of change of likelihood function with respect to K (ΔK) over
966 successive K values. Peak indicates modal value of ΔK distribution corresponding to optimal
967 K.

968

969 **Figure 3.** Population assignment of current and historical samples by STRUCTURE into
970 three genetically distinct populations (different shades of grey). Each individual represented
971 as separate vertical bar sectioned into shaded segments representing different genetic units.
972 Segment length is proportional to likelihood of assignment (proportion of times in 100,000
973 iterations) of individual to that population. Putative temporal populations shown as
974 1=historical, 2=current separated by black line, although this information was not used *a*
975 *priori* for analysis.

976

977 **Figure 4.** Distribution of allele frequencies for historical and current populations across all
978 loci.

979

980 **Figure 5.** Bayesian estimates of historical and current N_e using TMVP: a) posterior
981 distribution of historical and current N_e, with density of points proportional to probability
982 density of N_e at time of oldest and youngest samples; b) 5%, 50% and 95% higher posterior
983 density limits of posterior distribution, and joint mode of historical and current N_e (single
984 solid circle).

Table 1. Genetic diversity for each neutral polymorphic microsatellite locus, and summary statistics (overall mean or total ±S.E.) of genetic diversity of current and historical populations across all loci. Diversity measures: number of alleles/locus (*Na*); number of unique alleles/locus (*Pa*); allelic richness (*Ar*); unique allelic richness (*Pr*); observed heterozygosity (H_o); expected heterozygosity (H_e). Inbreeding estimator (F_{IS}) was used to detect deviations from HWE for each locus and each population. Overall values (bold) represent population values: average *Na*, *Pa*, *Ar*, *Pr*, H_o, H_e, overall F_{IS}.

Locus	**Historical population (*n*=8)**							**Current population (*n*=9)**						
	Na	*Pa*	*Ar*	*Pr*	H_o	H_e	F_{IS}	*Na*	*Pa*	*Ar*	*Pr*	H_o	H_e	F_{IS}
D7S817	5	4	4.750	4.000	0.625	0.750	0.176	2	1	2.000	1.000	1.000	0.529	-1.000
DQCar	4	2	3.867	2.000	0.750	0.592	-0.292	2	0	2.000	0.008	0.889	0.523	-0.778
D1S548	3	0	3.000	0	0.875	0.692	-0.289	3	0	2.961	0	0.778	0.621	-0.273
HPRT1	3	1	3.000	1.000	0.286	0.670	0.593	2	0	2.000	0	0.222	0.366	0.407
D9S302	2	0	2.000	0	0.286	0.440	0.368	2	0	2.000	0	0.222	0.366	0.407
DXYS156	2	0	2.000	0	0.857	0.527	-0.714	2	0	1.778	0	0.111	0.111	0.000
D5S1470	3	2	3.000	2.000	0.571	0.615	0.077	2	1	1.995	0.995	0.333	0.294	-0.143
DXS8043	3	1	3.000	1.000	0.429	0.692	0.400	2	0	2.000	0	0.444	0.471	0.059
D6S265	5	1	4.867	1.000	0.500	0.767	0.364	4	0	3.956	0.125	1.00	0.725	-0.412
D2S367	4	3	3.875	3.000	0.500	0.717	0.317	2	1	2.000	1.000	1.00	0.529	-1.000
D5S1457	3	1	2.875	1.00	0.375	0.425	0.125	2	0	1.961	0	0.222	0.209	-0.067
Overall	**3.364**	**1.364**	**3.290**	**1.340**	**0.550**	**0.626**	**0.129**	**2.273**	**0.273**	**2.240**	**0.280**	**0.566**	**0.431**	**-0.337**
S.E.	0.310	0.388	0.288	0.358	0.063	0.036	n/a	0.195	0.141	0.194	0.139	0.111	0.055	n/a

Table 2. Results from heterozygosity excess tests in BOTTLENECK using three significance tests and four models of allele mutation (IAM, SMM, two TPM variants). Significant *P*-values (<0.05) indicated in bold.

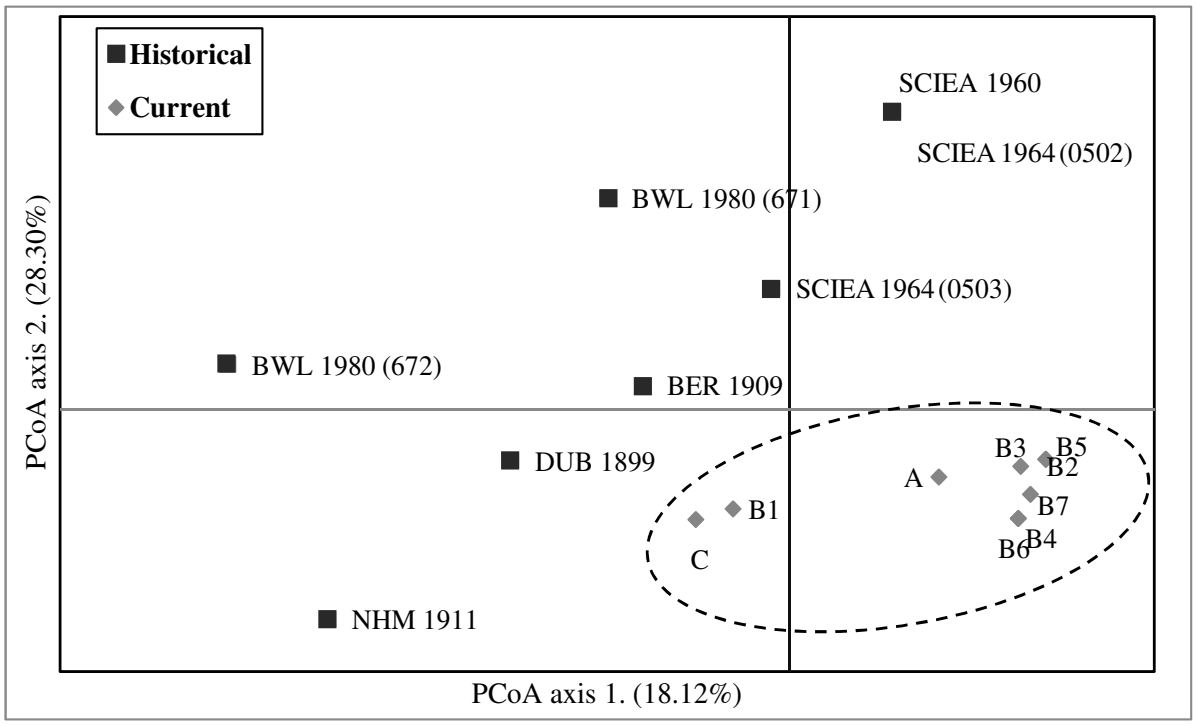
Test	Test statistic/probability variant	Historical population				Current population			
		IAM	SMM	TPM (70% SMM)	TPM (90% SMM)	IAM	SMM	TPM (70% SMM)	TPM (90% SMM)
Sign test	Expected number loci with heterozygosity excess	6.16	6.50	6.36	6.60	5.66	5.72	5.29	5.56
	Observed number loci with heterozygosity excess	9	8	9	9	9	8	8	8
	Probability (*P*-value)	0.073	0.274	0.092	0.118	**0.040**	0.140	0.090	0.119
Standardised differences test	T2	2.65	1.15	1.92	1.54	2.32	1.37	1.75	1.54
	Probability (*P*-value)	**0.0040**	0.124	**0.028**	0.062	**0.010**	0.086	**0.040**	0.061
Wilcoxon sign-rank test	Probability (one tail for heterozygosity deficiency)	0.999	0.926	0.992	0.966	0.994	0.913	0.966	0.966
	Probability (one tail for heterozygosity excess)	**0.001**	0.087	**0.011**	**0.042**	**0.008**	0.103	**0.042**	**0.042**
	Probability (two tails for heterozygosity excess/deficiency)	**0.002**	0.175	**0.021**	0.083	**0.016**	0.206	0.083	0.083

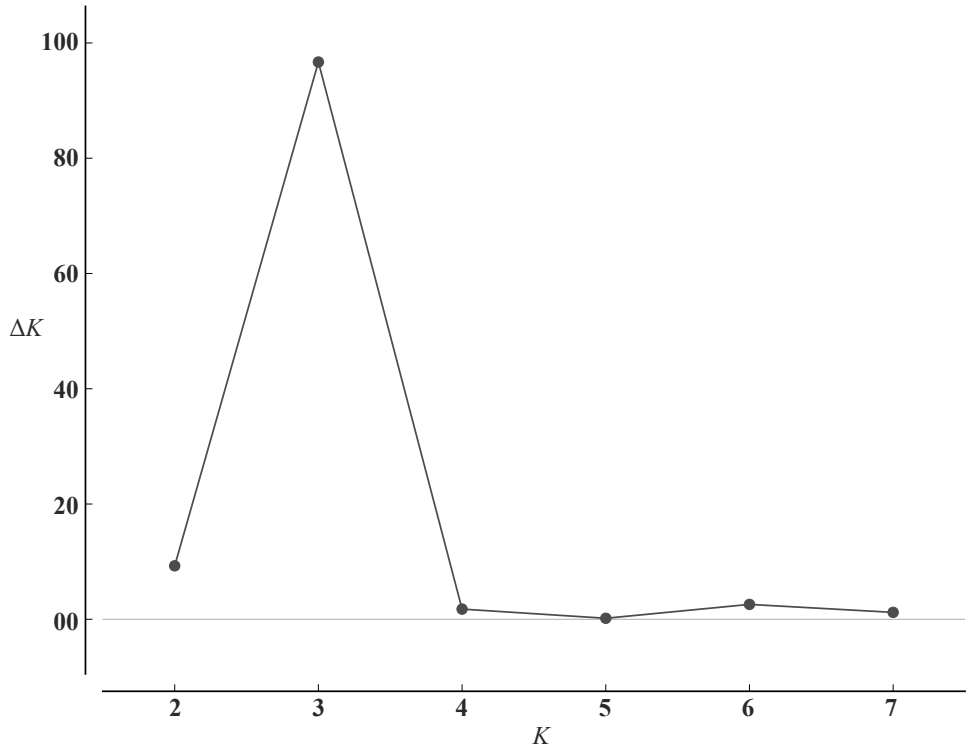
Table 3. N_e estimates of current and historical populations inferred via linkage disequilibrium (LD), heterozygosity excess (HE), molecular coancestry (Coan.), and full-likelihood sib-ship assignment (FL); 95% confidence intervals in parentheses.

Population	**LD (95% CI)**	**HE (95% CI)**	**Coan. (95% CI)**	**FL (95% CI)**
Historical	2.8 (1.5-16.5)	Infinity (8.5-infinity)	4.4 (2.1-7.6)	16 (7.0-86)
Current	3.1 (1.2-infinity)	2.6 (1.4-infinity)	1.8 (1.0-2.9)	4 (2.0-20)

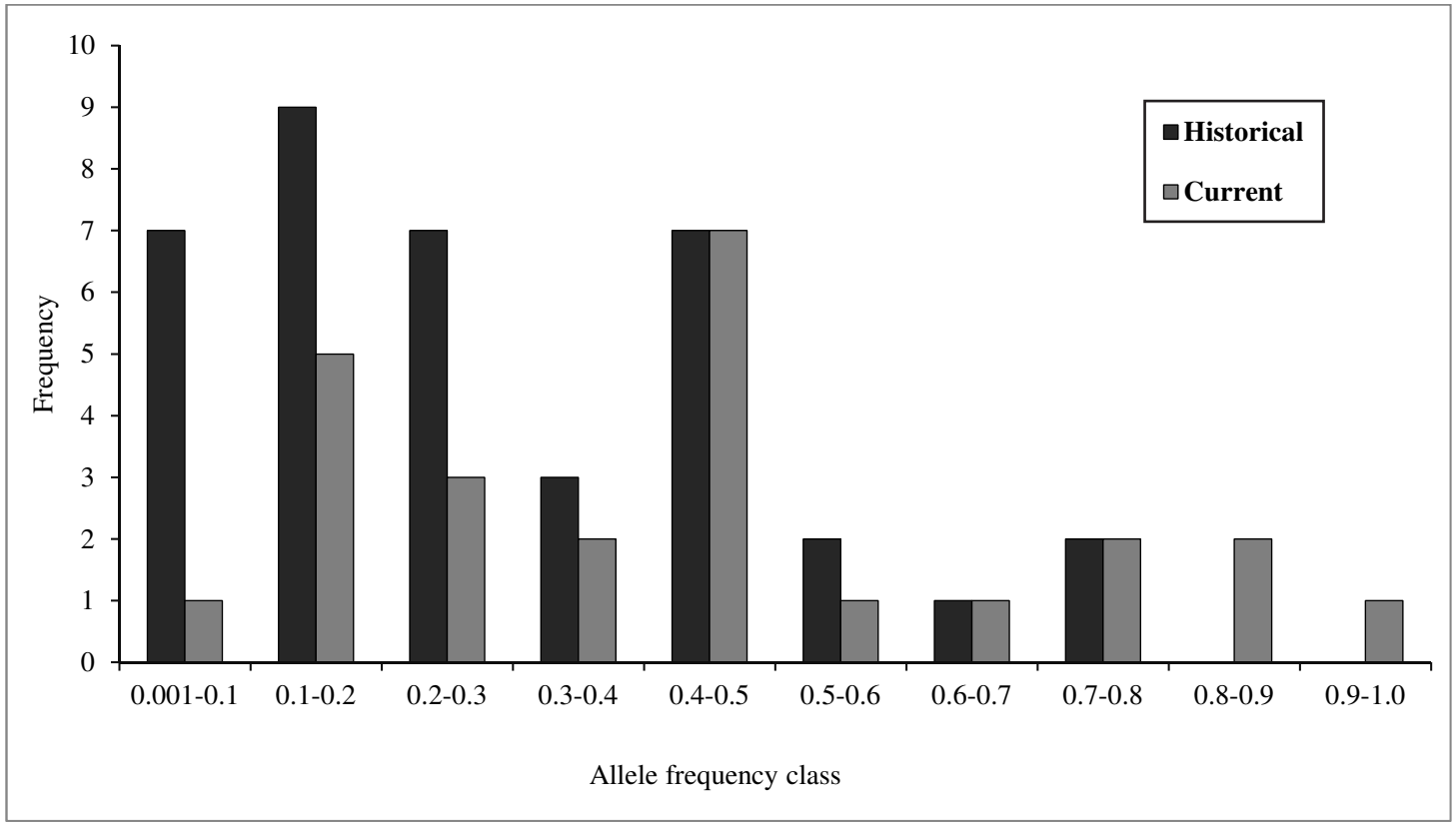
Table 4. Sex ratios for current population and results of tests for deviation from 1:1 sex ratio using Yates-corrected Pearson's Chi-squared statistic. Degrees of freedom for all tests=1; sample size used to calculate ratios indicated.

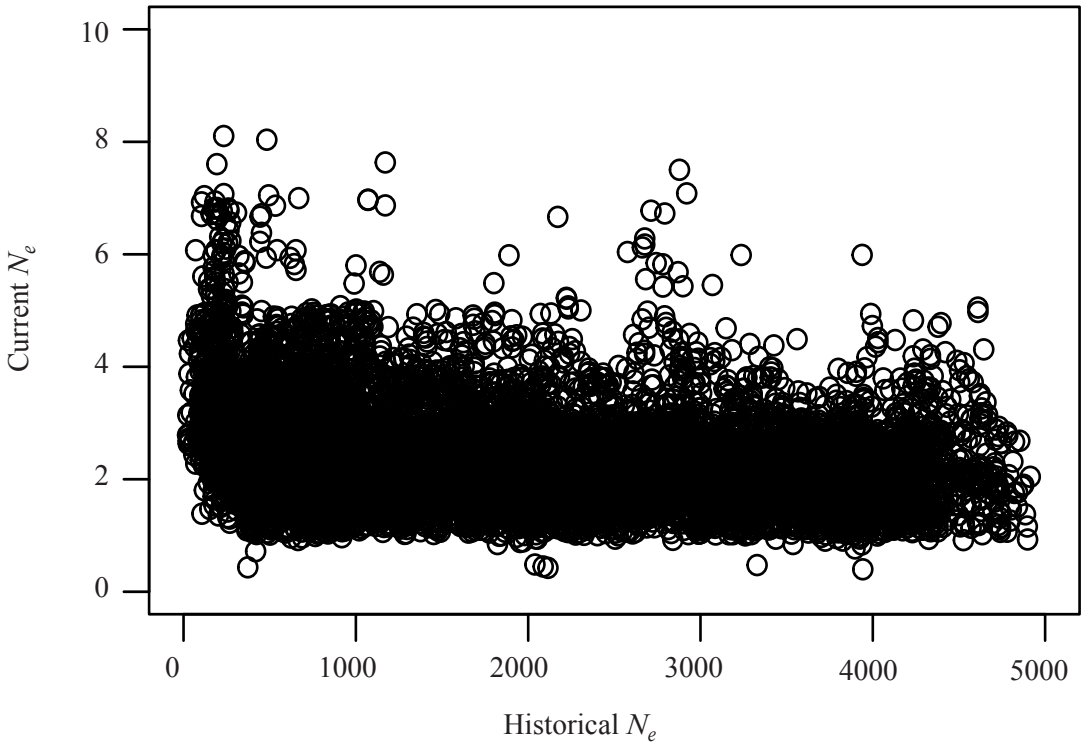
Sex ratio tested	**Females**	**Males**	**Observed ratio (female:male)**	**Yates-corrected Chi-square test (χ2) against 1:1**	
Sampled population (*n*=9)	3	6	1 : 2	Yates χ2	0.44
				P	0.50
Group B (*n*=7)	3	4	3 : 4	Yates χ2	0
				P	1
Group B offspring (*n*=4)	1	3	1 : 3	Yates χ2	0.25
				P	0.62


Historical
Current
SCIEA 1960
SCIEA 1964 (0502)
BWL 1980 (671)
SCIEA 1964 (0503)
BWL 1980 (672)
BER 1909
DUB 1899
NHM 1911
A
B1
B2
B3
B4
B5
B6
B7
C
PCoA axis 2. (28.30%)
PCoA axis 1. (18.12%)

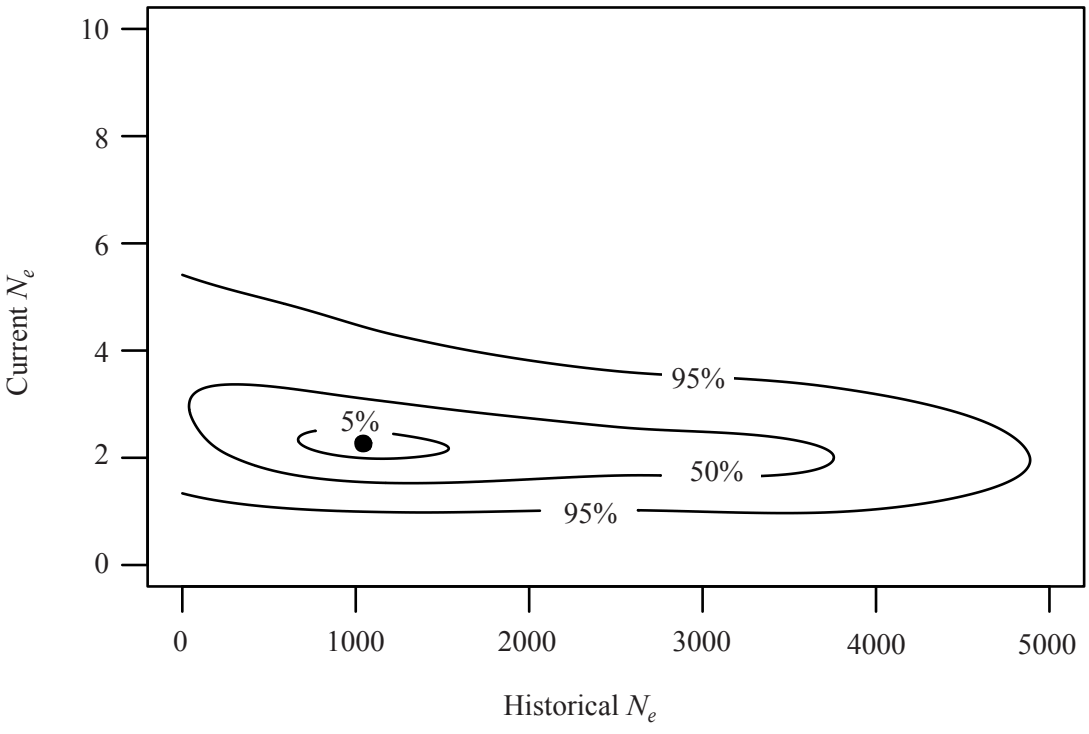

100
80
60
ΔK
40
20
00
2
3
4
5
6
7
K


Cluster assignment
1.00
0.80
0.60
0.40
0.20
0.00
1
2
Putative temporal populations


10
9
8
7
6
5
4
3
2
1
0
Frequency
Historical
Current
0.001-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0
Allele frequency class

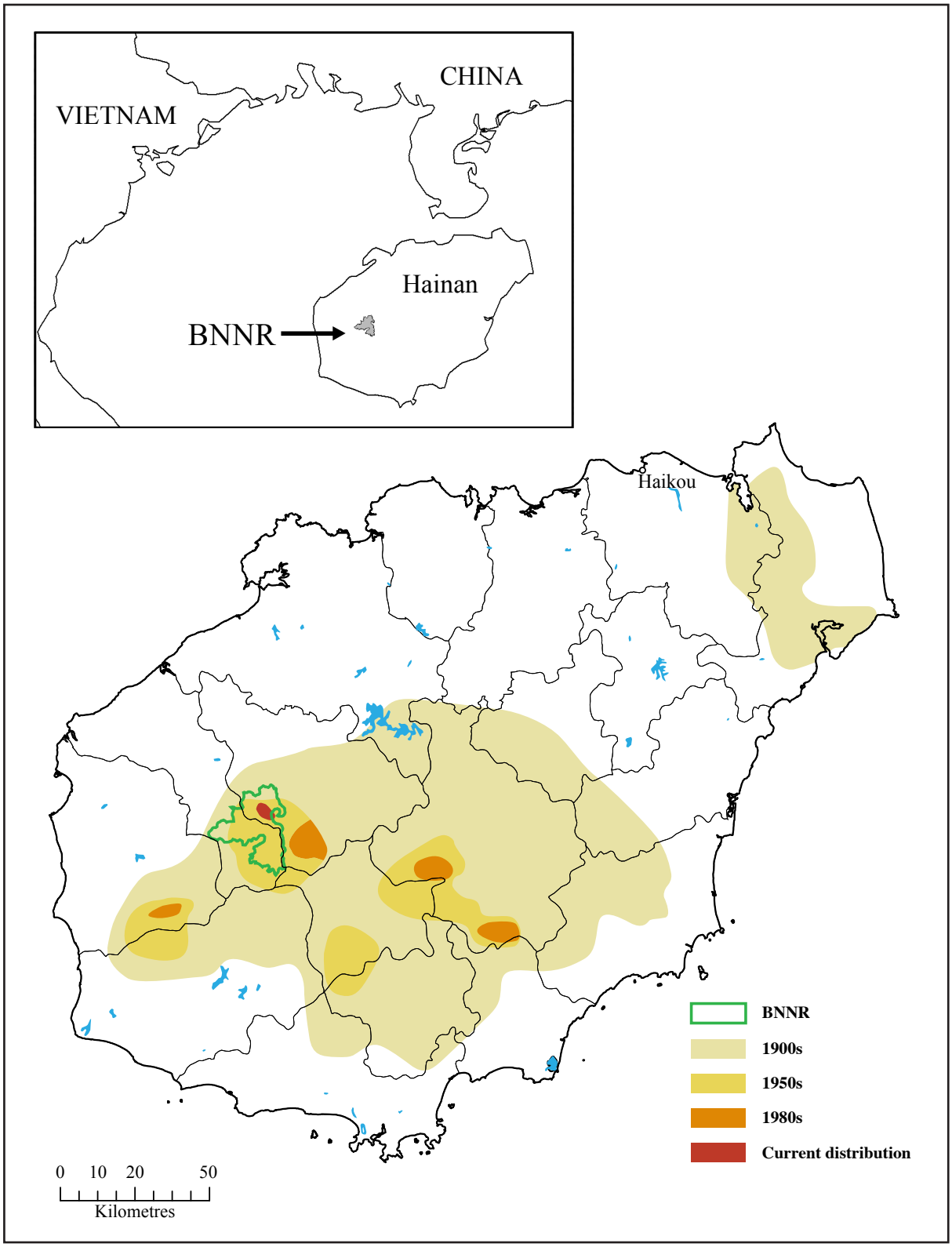

10
8
6
4
2
0
Current N_e
0
1000
2000
3000
4000
5000
Historical N_e

a)


10
8
6
4
2
0
Current N_e
95%
5%
50%
95%
0
1000
2000
3000
4000
5000
Historical N_e

b)

Appendix S1. Map showing the location of the sole surviving population of the Hainan gibbon (*Nomascus hainanus*), which is constrained to Bawangling National Nature reserve (BNNR) on Hainan Island, China. This current global distribution is also contextualised within the inferred historical distribution across Hainan, illustrating the species' drastic range contraction from 1900 to its current highly limited distribution (after Chan et al. 2005 and Zhou et al. 2005).



Appendix S2. Existing museum specimens of *Nomascus hainanus* sampled for assessment of species' historical genetic state, with details of samples collected.

Year	Museum/Collection	Specimen accession number	Species (as listed in museum catalogue)	Locality information	Specimen type	Sample type
1899	National Museum of Ireland, Dublin	NMINH:1899.51.1	*Hylobates hainanus*	China	skin (mounted specimen) and skull	skin tissue and bone fragments
1891	Natural History Museum, London	ZD.1891.12.10.1	*Hylobates concolor hainanus*	Hainan [19°00' N, 109°30' E]	skin (mounted specimen) ***Holotype***	***SAMPLING NOT PERMITTED***
1893	Natural History Museum, London	ZD.1893.9.12.1	*Hylobates concolor hainanus*	Hainan [19°00' N, 109°30' E]	skin and skull (and skeleton)	skin tissue
1907	Natural History Museum, London	ZD.1907.12.1.1	*Hylobates concolor hainanus*	Hainan [19°00' N, 109°30' E]	skin	skin tissue
1911	Natural History Museum, London	ZD.1911.2.24.4	*Hylobates concolor hainanus*	Hainan [19°00' N, 109°30' E]	skin and skull	skin tissue
1909	Museum für Naturkunde, Berlin	Inv. No. 84622	*Nomascus concolor hainanus*	Hainan	skin and skull	skin tissue
1909	Museum für Naturkunde, Berlin	Inv. No. 85357	*Nomascus concolor hainanus*	Hainan ("Hoi Chow")	skin	skin tissue
1962	South China Institute of Endangered Animals, Guangzhou	0088	*Hylobates concolor hainanus*	Jianfengling, Hainan	skin and skull	hairs with residual skin tissue
1964	South China Institute of Endangered Animals, Guangzhou	0502	*Hylobates concolor hainanus*	Bawangling, Hainan	skin (mounted specimen) and skull	dried muscle tissue and bone fragments
1964	South China Institute of Endangered Animals, Guangzhou	0503	*Hylobates concolor hainanus*	Bawangling, Hainan	skin and skull	skin tissue and bone fragments
c. 1960s	South China Institute of Endangered Animals, Guangzhou	uncatalogued	unlabelled (identified as *N. hainanus*)	unknown, likely Bawangling	post-cranial bones	dried muscle tissue
1980	Haikou University/BNNR Management Office, Hainan	671	*Nomascus hainanus*	Bawangling, Hainan	skin (with skeleton)	skin tissue and bone fragments
1980	Haikou University/BNNR Management Office, Hainan	672	*Nomascus hainanus*	Bawangling, Hainan	skin (with skeleton)	skin tissue and bone fragments

Appendix S3. Details of human microsatellite loci screened. 'Method' refers to whether loci were tested only at screening phase (Qiaxcel) or fully genotyped (Sequenced). Where 'no amplification' is reported, the locus failed to produce products for all *N. hainanus* samples (although it may have amplified for control gibbon species). PCR annealing temperatures reported for failed loci represent the lowest temperatures tested.

Locus	Repeat motif	Previous use for gibbons	Gibbon species	Approximate product size (bp)	Sample type	Method	Annealing Temp. (°C)	No. PCR cycles	*N. hainanus* result
D1S207	Di	Clisson et al. (2000)	*Hylobates lar*	128	blood	Qiaxcel	50;48	15;20	*no amplification*
D1S548	Tetra	Chambers et al. (2004)	*Hylobates lar*	160-188	faecal	Qiaxcel, Sequenced	53	35	**polymorphic**
D1S550	Tetra	Chambers et al. (2004)	*Hylobates lar*	poor amplification	faecal	Qiaxcel, Sequenced	50	35	monomorphic
D2S1329	Tetra	Chambers et al. (2004)	*Hylobates lar*	188-216	faecal	Qiaxcel, Sequenced	50	35	monomorphic
D2S1777	Tetra	Oka & Takenaka (2001)	*Hylobates muelleri*	190-230	hair or faeces	Qiaxcel, Sequenced	46	35	*poor amplification*
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				
D2S367	Di	Oka & Takenaka (2001)	*Hylobates muelleri*	102-170	hair or faeces	Qiaxcel, Sequenced	54	30	**polymorphic**
		Chambers et al. (2004)	*Hylobates lar*	poor amplification	faecal				
D5S1457	Tetra	Chambers et al. (2004)	*Hylobates lar*	130-154	faecal	Qiaxcel, Sequenced	54	30	**polymorphic**
D5S1470	Tetra	Chambers et al. (2004)	*Hylobates lar*	poor amplification	faecal	Qiaxcel, Sequenced	50	35	**polymorphic**
D5S807	Tetra	Oka & Takenaka (2001)	*Hylobates muelleri*	142-190	hair or faeces	Qiaxcel, Sequenced	54	30	monomorphic
		Chambers et al. (2004)	*Hylobates lar*	poor amplification	faecal				
D6S265	Di	Clisson et al. (2000)	*Hylobates lar*	156	blood	Qiaxcel, Sequenced	56;54	15;20	**polymorphic**
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				
D6S2972/ MOG-CA	Di	Clisson et al. (2000)	*Hylobates lar*	136	blood	Qiaxcel	50;48	15;20	*no amplification*

D7S503	Di	Clisson et al. (2000)	*Hylobates lar*	130	blood	Qiaxcel	50;48	15;20	*no amplification*
D7S817	Tetra	Chambers et al. (2004)	*Hylobates lar*	poor amplification	faecal	Qiaxcel, Sequenced	56;54	15;20	**polymorphic**
D8S1106	Tetra	Chambers et al. (2004)	*Hylobates lar*	no amplification	faecal	Qiaxcel, Sequenced	46	35	*poor amplification*
D9S302	Tetra	Oka & Takenaka (2001)	*Hylobates muelleri*	180-230	hair or faeces	Qiaxcel, Sequenced	56;54	15;20	**polymorphic**
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				
D10S1432	Tetra	Chambers et al. (2004)	*Hylobates lar*	152-198	faecal	Qiaxcel, Sequenced	46	35	*poor amplification*
D11S1984	Tetra	Oka & Takenaka (2001)	*Hylobates muelleri*	150-220	hair or faeces	Qiaxcel, Sequenced	54	35	monomorphic
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				
D13S321	Tetra	Chambers et al. (2004)	*Hylobates lar*	215-251	faecal	Qiaxcel	*50*	35	*no amplification*
D14S255	Di	Oka & Takenaka (2001)	*Hylobates muelleri*	172-190	hair or faeces	Qiaxcel, Sequenced	56;54	15;20	*poor amplification*
D14S306	Tetra	Oka & Takenaka (2001)	*Hylobates muelleri*	142-172	hair or faeces	Qiaxcel, Sequenced	56;54	15;20	monomorphic
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				
D16S2624	Tetra	Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal	Qiaxcel, Sequenced	56;54	15;20	monomorphic
D17S804	Di	Oka & Takenaka (2001)	*Hylobates muelleri*	130-150	hair or faeces	Qiaxcel, Sequenced	54	35	**polymorphic**
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				
D20S206	Tetra	Oka & Takenaka (2001)	*Hylobates muelleri*	100-120	hair or faeces	Qiaxcel, Sequenced	53	35	**polymorphic**
		Chambers et al. (2004)	*Hylobates lar*	159-175	faecal				
DQcar	Di	Clisson et al. (2000)	*Hylobates lar*	108-117	blood	Qiaxcel, Sequenced	53	35	**polymorphic**
		Roeder et al. (2009)	*Hylobates lar*	107-117	muscle or hair				
		Crouau-Roy (1999)	*Hylobates lar*	107-117	*not reported*				
		Chambers et al. (2004)	*Hylobates lar*	monomorphic	faecal				

DXS571	Di	Roeder et al. (2009)	*Hylobates lar*	131-135	muscle or hair	Qiaxcel, Sequenced	56;54	15;20	monomorphic
DXS8043	Di	Roeder et al. (2009)	*Hylobates lar*	173-175	muscle or hair	Qiaxcel, Sequenced	56;54	15;20	**polymorphic**
DXYS156	Penta	Roeder et al. (2009)	*Hylobates lar*	116-126	muscle or hair	Qiaxcel, Sequenced	58	30	**polymorphic**
HPRT1	Tetra	Roeder et al. (2009)	*Hylobates lar*	144-156	muscle or hair	Qiaxcel, Sequenced	56;54	15;20	**polymorphic**
		Watanabe et al. (1997)	*Hylobates lar*	not reported	blood				
TNFa/b	Di	Clisson et al. (2000)	*Hylobates lar*	211	blood	Qiaxcel	*50*	35	*no amplification*
		Chambers et al. (2004)	*Hylobates lar*	poor amplification	faecal				
vWF	Tetra	Chambers et al. (2004)	*Hylobates lar*	no amplification	faecal	Qiaxcel	48	35	*no amplification*

Appendix S4. Thirteen successfully genotyped polymorphic microsatellite loci, showing final annealing temperatures used and characterisation in *N. hainanus* across all samples.

Locus	Annealing Temp. (°C)	Allele size range	Number of alleles
D1S548	53	161-173	3
D2S367	54	138-156	5
D5S1457	54	110-118	3
D5S1470	50	192-204	4
D6S265	56;54	118-134	5
D7S817	56;54	130-148	6
D9S302	56;54	188-192	2
D17S804	54	145-161	3
D20S206	53	132-144	2
DQcar	53	86-104	4
DXS8043	56;54	190-196	3
DXYS156	58	115-125	2
HPRT1	56;54	138-148	3

Appendix S5. Assessment of the impact of the incorporation of closely-related individuals upon population structure within the overall study sample

In cases where a large sample of individuals is obtained from a large, spatially dispersed population in which there may be hidden sub-population structure, the presence of closely related individuals can led to the detection of population genetic structure when it is absent or to the overestimation of the number of clusters (Rodríguez-Ramilo and Wang 2012). Given the close relationships observed for all all individuals in the current population sample, we wanted to be certain that the population structure observed was not purely the result of any such relationships.

Following the approach of Rodríguez-Ramilo et al. (2014), we investigated the extent and pattern of clustering within our overall sample (current and historical samples combined) using the CLUSTER_DIST program, which maximizes the genetic distances between groups and thereby avoids the assumptions of Hardy-Weinberg and linkage equilibrium. We ran the cluster assignment analysis using a range values for the possible number of clusters (K=2-8) and then used the ΔK calculation outlined in Rodríguez-Ramilo et al. (2009) to assess the rate of change in the averaged genetic distance between successive K values. This indicated that the inferred number of clusters (corresponding to the value with the highest ΔK) in our overall sample was K=6 (Figure S1). Despite this higher number of clusters compared to our STRUCTURE result (K=3), the overall pattern of clustering (based upon the highest probability cluster assignment for each sample) matched that of the PCoA analysis closely, and did not differ dramatically from the STRUCTURE result in terms of the clustering of the current samples: most of the current samples (7 of 9 individuals) were still grouped into one cluster (Table S1). In line with the results from the STRUCTURE analysis, two current population samples (B1 and C) were again

grouped with some of the older samples (NHM 1911 and BWL 1989 (672)), supporting our STRUCTURE result and again suggesting a small amount of historical genetic structure may persist in the current population, contained within genotypes of these two individuals. The CLUSTER_DIST analysis also revealed some finer-scale population subdivision within the historical population sample, identifying additional clusters that largely correspond to temporal divisions of the historical sample (Table S1). However, as the results of this analysis generally supported the original STRUCTURE and PCoA results by maintaining the grouping the majority of the current samples into one cluster/population, it is unlikely that the pattern of the majority of current samples being grouped together is solely due to the close relationships between these individuals. This indicates that our original STRUCTURE analysis and its results regarding population differentiation between the current and historical samples are valid, despite the close relationships between individuals sampled from the current population.

References

Rodríguez-Ramilo ST, Toro MA, Fernández J (2009) Assessing population genetic structure via the maximisation of genetic distance. *Genetics Selection Evolution*, **41**, 49.

Rodríguez-Ramilo ST, Wang J (2012) The effect of close relatives on unsupervised Bayesian clustering algorithms in population genetic structure analysis. *Molecular Ecology Resources*, **12**, 873-884.

Rodriguez-Ramilo ST, Toro MA, Wang J *et al.* (2014) Improving the inference of population genetic structure in the presence of related individuals. *Genetics Research*, **96**, e003.

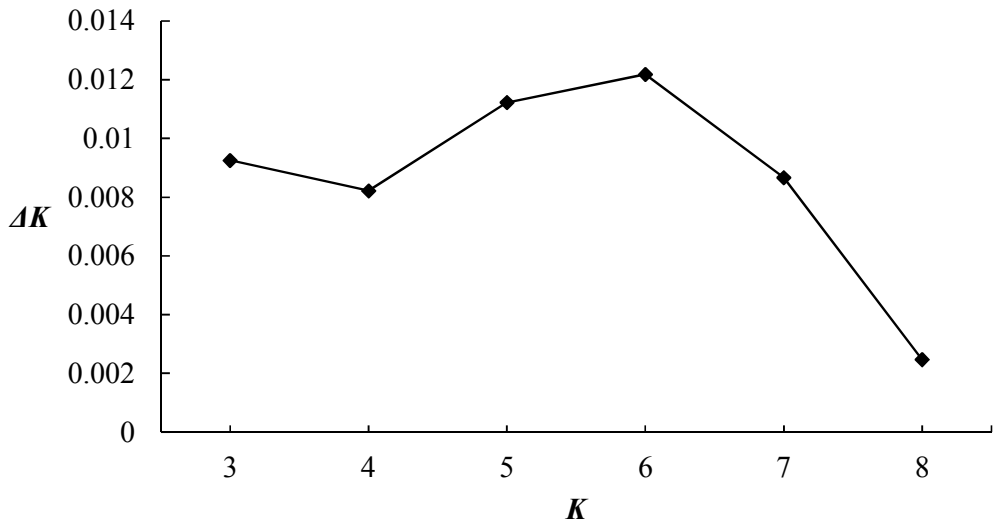


Figure S1. Rate of change in the average genetic distance between successive *K* values (Δ*K*) over increasing *K* values (calculated as per equation in Rodríguez-Ramilo et al. 2009). The inferred number of clusters corresponds to the value with the highest Δ*K* (*K*=6).

Table S1. Cluster assignment of all samples (current and historical) by CLUSTER_DIST for inferred number of clusters *K*=6, based upon highest cluster probabilities (highlighted in **bold**). Putative temporal populations also shown, although this information was not used within the analysis.

Putative Population	Sample	Highest Probability Cluster	CLUSTERS					
			1	*2*	*3*	*4*	*5*	*6*
Historical	DUB 1899	*5*	0	0.01	0.01	0	**0.96**	0.01
	BER 1909	*3*	0.01	0	**1**	0	0	0
	SCIEA 1964 (0503)	*1*	**0.97**	0.01	0.01	0	0	0.01
	SCIEA 1964 (0502)	*1*	**0.97**	0	0.01	0.01	0.01	0
	SCIEA 1960	*1*	**0.98**	0	0	0.01	0	0.01
	BWL 1980 (671)	*6*	0	0	0	0	0	**1**
	NHM 1911	*4*	0.01	0	0	**0.99**	0	0
	BWL 1980 (672)	*4*	0.01	0.01	0	**0.98**	0	0
Current	B1	*4*	0.01	0	0.01	**0.98**	0	0
	C	*4*	0	0	0.01	**0.99**	0	0
	A	*2*	0	**0.98**	0	0.01	0.01	0.01
	B2	*2*	0.01	**0.97**	0.01	0.01	0	0
	B4	*2*	0	**1**	0	0.01	0	0
	B5	*2*	0.01	**0.98**	0.01	0	0	0
	B6	*2*	0	**0.99**	0	0	0.01	0.01
	B3	*2*	0	**0.97**	0.01	0	0.01	0
	B7	*2*	0.01	**0.98**	0.01	0	0	0

Appendix S6. Tests of linkage disequilibrium between eleven successfully genotyped polymorphic microsatellite loci pairs, within each temporal population and across all samples. Significant *P*-values ($P<0.05$) indicated in **bold** and marked with *****. (Note: no pairs showed significance at a more conservative Bonferroni-corrected 5% *P*-value, $P=0.000455$).

Locus pair		Current population	Historical population	ALL
D1S548	HPRT1	0.17273	0.83636	0.24545
D1S548	D9S302	0.17091	0.86409	0.30409
D1S548	DXYS156	0.45364	1.00000	0.60682
D1S548	D5S1470	0.17318	0.09366	0.07899
D1S548	DXS8043	0.20545	0.79091	0.30591
D1S548	D6S265	0.90091	**0.04364***	0.20136
D1S548	D2S367	0.95390	0.92773	0.92490
D1S548	D5S1457	0.06045	0.75864	0.17688
D2S367	D5S1457	0.16268	0.18500	0.17343
D5S1470	DXS8043	1.00000	0.72773	0.90497
D5S1470	D6S265	0.20955	0.10818	0.17881
D5S1470	D2S367	0.77896	0.58727	0.66718
D5S1470	D5S1457	0.50864	1.00000	0.82227
D6S265	D2S367	0.51889	0.48227	0.49264
D6S265	D5S1457	1.00000	0.41182	0.75682
D7S817	DQCar	0.12864	0.10409	0.11121
D7S817	D1S548	0.38945	0.43591	0.41635
D7S817	HPRT1	0.37173	0.36455	0.36651
D7S817	D9S302	0.59822	0.56727	0.58032
D7S817	DXYS156	1.00000	1.00000	1.00000
D7S817	D5S1470	0.76881	0.69591	0.71644
D7S817	DXS8043	0.77987	0.73364	0.74630
D7S817	D6S265	0.49964	0.50727	0.50055
D7S817	D2S367	0.51637	0.49227	0.50809
D7S817	D5S1457	0.89476	0.93636	0.92608
D9S302	DXYS156	0.33636	0.14864	0.19490
D9S302	D5S1470	1.00000	0.84136	0.88273
D9S302	DXS8043	0.05159	0.36773	0.13571
D9S302	D6S265	0.66909	0.75500	0.59227
D9S302	D2S367	0.78736	0.74682	0.77896
D9S302	D5S1457	0.09000	0.15000	0.13309
DQCar	D1S548	1.00000	0.13227	0.18402
DQCar	HPRT1	0.31318	0.48318	0.20727
DQCar	D9S302	0.32818	0.10227	0.16056
DQCar	DXYS156	1.00000	0.47818	0.53409
DQCar	D5S1470	0.33500	0.31179	0.34032
DQCar	DXS8043	1.00000	0.75773	0.78273

DQCar	D6S265	0.21864	0.58045	0.13227
DQCar	D2S367	0.48695	0.58318	0.57947
DQCar	D5S1457	1.00000	1.00000	1.00000
DXS8043	D6S265	0.29636	0.12773	0.08136
DXS8043	D2S367	0.27982	0.13455	0.23221
DXS8043	D5S1457	0.23000	0.34864	0.16455
DXYS156	D5S1470	1.00000	1.00000	1.00000
DXYS156	DXS8043	1.00000	1.00000	1.00000
DXYS156	D6S265	1.00000	1.00000	1.00000
DXYS156	D2S367	0.57947	0.36227	0.56449
DXYS156	D5S1457	0.22818	0.28659	0.23798
HPRT1	D9S302	**0.00364***	0.59773	**0.03187***
HPRT1	DXYS156	0.33636	0.38945	0.33695
HPRT1	D5S1470	1.00000	0.47500	0.60636
HPRT1	DXS8043	**0.04864***	0.07294	**0.02073***
HPRT1	D6S265	0.69364	0.39636	0.39984
HPRT1	D2S367	0.65449	0.39318	0.50761
HPRT1	D5S1457	0.08442	0.30026	0.11850

Appendix S7. Population pedigree of current Hainan gibbon population

The ML configuration was used to construct a pedigree for the current population within Pedigree Viewer V.6.5B (Kinghorn and Kinghorn 2010). All sampled mature individuals were included as candidate parents in the derivation of the ML configuration, regardless of current social group affiliations. Known relationships were incorporated where sufficient demographic data were available from long-term field observations. Where a likely parent was not represented in specified candidate adults, they were inferred from observed offspring genotypes (represented as starred and dashed numbers). An arbitrary genotyping error rate of 0.01 was imposed, with low error assumed due to the conservative genotyping approach adopted, and 50% probability that a given offspring's actual father or mother was included in candidate datasets. Inbreeding was incorporated into the model but did not alter the configuration produced.

The ML configuration of relationships for the current population (Figure S2) replicated known Group B parent-offspring associations incorporated *a priori* (e.g. B2 assigned maternity of B4-B7; B1 assigned maternity of B2), and revealed additional within-group relationships, including some suggested but not confirmed by field observations and not included *a priori* (e.g. B3 assigned paternity of B7). Paternities of older Group B offspring (B4-B6) were assigned to a theoretical male (*2), reflecting either sampling limitations or a true change in breeding male (from *2 to B3) between conception of B6 and B7, for which there is anecdotal field evidence. This theoretical male was also allocated paternities of the current Group B breeding pair (B2-B3), which would indicate inbreeding

within the group: B2 mating with her own father to produce B4-B6; and B2-B3 as siblings producing B7.

ML pairwise *r* estimates supported many of these Group B relationships (Table S2). Full-sibling relationships between Group B offspring (B4-B7) were evident ($r \geq 0.5$ for each pairwise comparison of these individuals), except for B5 and B7, which was slightly lower ($r=0.32$) but still greater than half-sibship. Parent-offspring level relationships were supported between B2 and her known offspring B4-B6 ($r \geq 0.5$), except B7 ($r=0.32$), which is still greater than half-sibship. Pairwise coefficients between B3 and Group B offspring supported parent-offspring relationships ($r \geq 0.5$), except for B5, which was closer to the half-sibship ($r=0.27$).

A maternal half-sib relationship was inferred between B3 and the adult male sampled from Group A (by #1; Figure S2), although ML pairwise *r* supported a closer, full-sib relationship ($r=0.5$). Adult males from Groups A and C were revealed as paternal half-sibs (by *1) or closer, with $r=0.38$ supporting between full and half-sibs. The oldest living female (B1) was allocated maternity of the adult male sampled from Group C, with $r>0.5$ supporting a parent-offspring relationship between these individuals.

To further inform this analysis, we also re-ran the COLONY and ML-RELATE analyses incorporating the two youngest specimens (BWL 671-672) from the historical population, collected from the Bawangling area in the 1980s. The eldest individual in the current population (B1) is estimated to be anywhere up to 45 years old (Li et al. 2010), and

47 therefore may be of the same cohort as these specimens, making it informative to assess
48 relatedness between these samples and the current population. When BWL 671-672 were
49 incorporated, both the ML configuration (Figure S3) and ML *r* estimate (Table S2; *r*=0.17)
50 indicated a roughly half-sib relationship between one of these historical individuals (BWL
51 672) and B1. The pedigree indicated this may have been the result of a shared mother
52 (#2). There was also limited evidence for an affiliation between BWL 672 and the Group
53 C adult male (*r*=0.13, approximately cousins), although this was not supported by the ML
54 configuration. The two historical individuals appear to have been between full and half-
55 sibs (*r*=0.35), likely the result of a shared father (*3). All relationships observed for the
56 current population under the original configuration were preserved.

57

58 **References**

59 Kinghorn B, Kinghorn S (2010) *Pedigree Viewer (version 6.5b)*. University of New
60 England, Armidale, Australia.

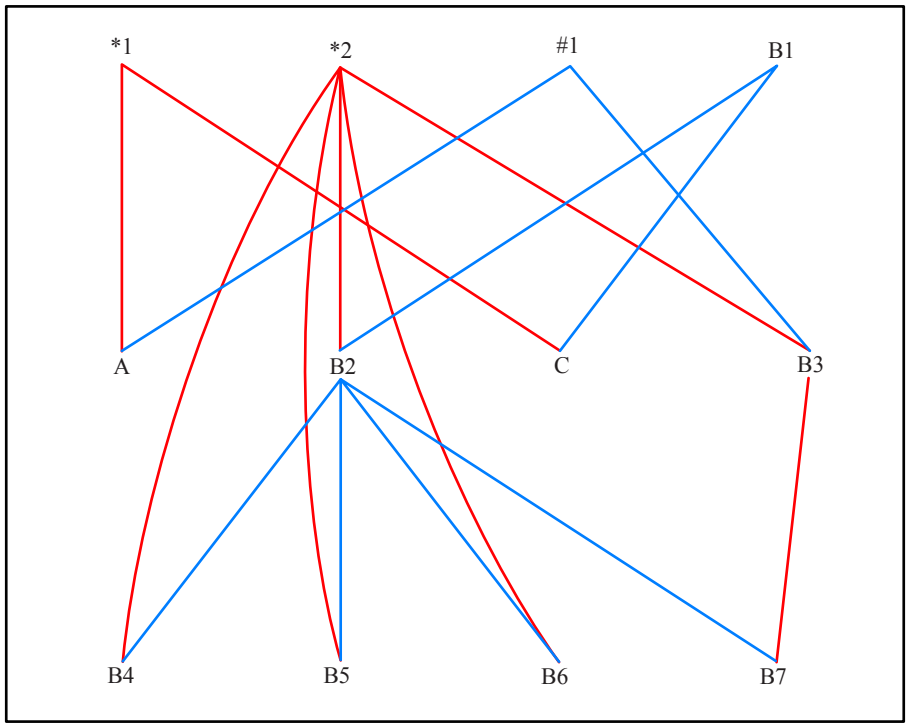
61 Li Z, Wei F, and Zhou J (2010) Mitochondrial DNA D-loop sequence analysis and
62 population rejuvenation of Hainan gibbons (*Nomascus hainanus*). *Biodiversity Science*, **18**,
63 523–552.

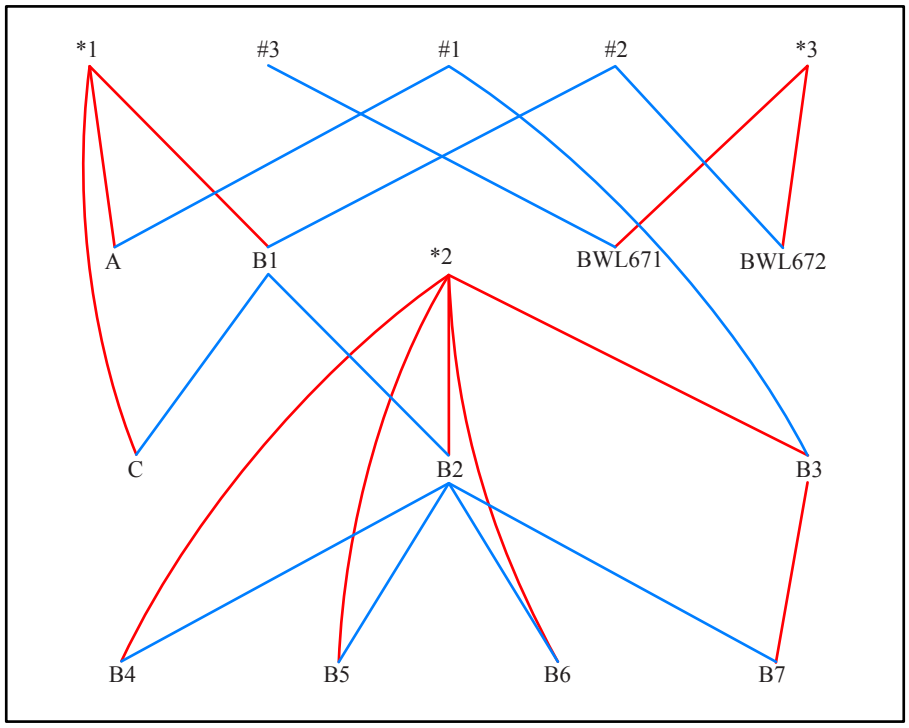
Table S2. ML relatedness coefficients (*r*) indicating pairwise relatedness between all sampled individuals of current population, and between these individuals and the two youngest historical specimens (BWL 671-672). Increasing values of *r* from cousins (0.125) to level of parent-offspring or full-siblings (0.5) indicated by increasing intensity of grey shading. Social groups outlined in bold boxes; historical samples separated from current samples by dashed lines.

	A	B1	B2	B3	B4	B5	B6	B7	C	BWL671	BWL672
A	1										
B1	0.08	1									
B2	0.15	0.05	1								
B3	0.50	0	0.27	1							
B4	0	0.09	0.63	0.55	1						
B5	0.15	0.05	0.85	0.27	0.63	1					
B6	0	0.09	0.63	0.55	0.88	0.63	1				
B7	0.27	0.01	0.32	0.84	0.76	0.32	0.76	1			
C	0.38	0.59	0.25	0	0	0.25	0	0	1		
BWL671	0	0	0	0	0	0	0	0	0	1	
BWL672	0	0.17	0	0	0	0	0	0	0.13	0.35	1

Figure S2. Pedigree constructed from ML configuration of relationships between individuals in current population. Red lines correspond to paternal lines; blue lines correspond to maternal lines. Probable (theoretical) fathers and mothers not sampled in study but inferred by COLONY based upon observed genotypes of sampled individuals are represented as starred (*) and hashed (#) numbers respectively.

Figure S3. Pedigree constructed from ML configuration of relationships between individuals in current population and youngest historical specimens (BWL 671-672). Caption otherwise as for Figure S2.


*1
*2
#1
B1
A
B2
C
B3
B4
B5
B6
B7


*1
#3
#1
#2
*3
A
B1
BWL671
BWL672
*2
C
B2
B3
B4
B5
B6
B7