COMMENTARY

Phylogeography, Population Genetics, and Conservation of Javan Gibbons (*Hylobates moloch*)

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Background

The island of Java is heavily populated (density of >1000 people/km²) and natural forest is found only scattered on many of the higher mountains. Forest loss reached its peak between 1850 and 1900, and although conversion and degradation have steadily reduced the available forest further, the pattern of forest fragmentation has remained fairly stable since (Koorders 1912; Whitten *et al.* 1996). As such, forest-dependent species, including the endemic Javan gibbon (*Hylobates moloch*), have been confined to isolated forest fragments for longer periods than many tropical species (Smith *et al.* 2017). This pattern of long-term fragmentation and isolation; the presence of relatively large (>500 individuals), intermediate (50–500 individuals), and small (<50 individuals) populations; and their extreme dependence on closed-canopy forest make the Javan gibbon a good model to study the population (conservation) genetics of tropical forest-dwelling species in a changing landscape.

Kheng *et al.* (2018) recently presented data on the phylogeography and population genetic structure of Javan gibbons, collating data from three different studies; one from the late 1990s and two from the early 2000s, with a total of 47 gibbons. They group the samples into six areas: Ujung Kulon (UK, area inhabited by gibbons ca. 85 km²), Mt. Halimun (HLM, ca. 270 km²), Mt. Salak (SLK, ca. 70 km²), Mt. Gede–Pangrango (GP, ca. 80 km²), Mts. Masigit–Simpang–Tilu

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(MST, ca. 260 km²), and Mt. Slamet (SLMT, ca. 40 km²) (Fig. 1). We refer to these areas by their abbreviations when discussing them specifically in the context presented by Kheng *et al.* (2018) but spell out their names when we discuss them in a different context. Kheng *et al.* (2018) reported support for a western (UK–HLM–SLK–GP) and a central (MST–SLMT) Evolutionary Significant Unit (ESU), with significant population structuring in the former but not in the latter. Genetic diversity (mtDNA d-loop haplotypes, nDNA microsatellite alleles, etc.) differed greatly between areas, with relatively low values for UK and SLK, but high values for HLM and MST. There was no clear relationship between genetic diversity and estimated gibbon population size or remaining forest area.

We have concerns regarding Kheng *et al.*'s (2018) sampling and analysis strategies, which, when taken into consideration, result in a distorted view of the phylogeography and population genetics structure of the Javan gibbon. Adding available information about the sampling locations shows that twice the number of areas were sampled than in the analysis of Kheng *et al.* (2018), that there is a clear case of Isolation by Distance (IBD), and that there is no support for multiple ESUs.

Fig. 1 Western Java showing areas of natural forest (in dark gray), roughly coinciding with the remaining distribution of Javan gibbons (*Hylobates moloch*), and the 12 areas from where the gibbon samples used by Kheng *et al.* (2018) originated. Numbers are the maximum number of gibbons included in their genetic analysis. The six circles represent the *a priori* populations named by Kheng *et al.* (2018), from west to east: Ujung Kulon UK, Mt. Halimun HLM, Mt. Salak SLK (the smaller circle included in HLM), Mt. Gede Pangrango GP, Mts. Masigit–Simpang–Tilu MST, and Mt. Slamet SLMT. Insert shows Isolation by Distance, i.e., the relationship between genetic distance (residuals relative to the mean, $\mu = 0$) and geographic distance (in km) between sampling locations for 35 Javan gibbons. For smaller forest areas without a site name we used the midpoint of the forest as the sampling location.

Genetic Variability at the Population Level

Kheng *et al.* (2018) state that the exact sampling location was unknown for 21 of the 47 samples, although the sampling area was known. In fact, these locality data are available in an earlier paper, first presented in 1997 (Supriatna *et al.* 1999), thus contradicting the statements of Kheng *et al.* (2018) that the samples were collected in 1999. The CITES permit linked to the import of these Javan gibbon samples into the United States (Andayani *et al.* 2001; Kheng *et al.* 2018) was used in or before 1996. Supriatna *et al.* (1999) predominantly uses the same data set as Kheng *et al.* (2018) and provides the pet names, sample locations, and genetic analyses for 15 of the 21 unidentified gibbons. This, with additional locality data available on the NCBI GenBank website, allows us to match individual genetic samples to their sampling locations, which in turn allows us to consider IBD (Meirmans 2012).

The *a priori* grouping of samples into six areas by Kheng *et al.* (2018) is also problematic; some areas are connected to each other and should be treated as a single area, whereas other areas consist of widely separated localities, with huge stretches of deforested and heavily developed land between them that are clearly impassable by forest-dependent gibbons. Specifically:

- a). Samples from HLM comprise gibbons from Mts. Halimun–Salak National Park (Ciguha, Cimalang, Kenteng) and from Bayah, Pelabuhan Ratu, and Jampang regencies south and southeast of Halimun (Fig. 1). All these regencies have isolated lowland forest areas including several with gibbons and are separated from the forest of Mts. Halimun–Salak by tens of kilometers of cultivated and developed land. Their isolation from the Mts. Halimun–Salak forests was already very pronounced in 1891 (Koorders 1912). None of these isolated forests cluster with Halimun, and Jampang is equidistant from Mts. Halimun–Salak, Mt. Gede–Pangrango, and Mt. Tilu.
- b). GP contains samples from Mt. Gede–Pangrango and Telaga Warna–Megamendung, but these areas have been isolated by the heavily developed Puncak Pass for decades.
- c). Samples from MST are indeed from Mt. Masigit (Cirata) and Mt. Simpang (Cidaun, Sindangbarang) but also from Mt. Papandayan (Margamulia), ca. 30 km to the east of Mt. Simpang. The forest on Mt. Masigit has been isolated from Mt. Simpang and Mt. Papandayan since at least 1891 (Koorders 1912).
- d). One of the two samples from SLMT is indeed from Mt. Slamet (Purbalingga) but the other is from Mts. Dieng (Linggoasri), ca. 40 km to the east. The forests on Mt. Slamet and Mts. Dieng were continuous in 1891, but were clearly separated by 1963 (van Steenis and Schippers-Lammertse 1965).
- e). Conversely, the forests on Mt. Halimun and Mt. Salak, both part of Mts. Halimun–Salak National Park, are still connected, with several gibbon groups present in the connecting forest corridor (Nijman 2015).

If we consider a gap of 20 km of nonforested land that has persisted for at least several decades sufficient to consider two populations of gibbons on either side of this gap as separate, then Kheng *et al.* (2018) sampled not six but seven populations with a very different composition of sampled gibbons. In that scenario Bayah, Halimun–Salak,

Pelabuan Ratu, Jampang, Gede–Pangrango, and Telaga Warna–Megamendung group together into one area, and Ujung Kulon, Mt. Masigit, Mt. Simpang, Mt. Papandayan, Mt. Slamet, and Mts. Dieng retain their status as isolated areas. Reducing the size of the gap to 3 km, which is still impassable for forest-living gibbons, suggests that Kheng *et al.* (2018) analyzed data from at least 12 populations: 1) Ujung Kulon, 2) Mts. Halimun–Salak, 3) Bayah, 4) Pelabuan Ratu, 5) Jampang, 6) Mt. Gede–Pangrango, 7) Telaga Warna–Megamendung, 8) Mt. Masigit, 9) Mt. Simpang, 10) Mt. Papandayan, 11) Mt. Slamet, and 12) Mts. Dieng. Using these 12 populations in the analysis rather than six has several ramifications for interpretation of the results presented by Kheng *et al.* (2018):

- a) The number of private haplotypes that were limited to single populations is underestimated (their Table II).
- b) Differences in population haplotype diversity are overestimated, as some of the most diverse areas comprise multiple isolated populations (their Table II).
- c) Area explains a lower level of molecular variance than Kheng *et al.* (2018) estimate (their Table V), because several of the areas comprise multiple isolated populations.
- d) Pairwise fixation index F_{st} and Jost's index of genetic differentiation D (their Table VI) change, because these depend on the identification of areas, and comparisons between areas that are in fact contiguous (parts of HLM and SLK) or that comprise multiple long-term isolated populations (MST) are not meaningful.
- e) The assumption of 6 populations by Kheng *et al.* (2018), whereas realistically there were 12, make it difficult to meaningfully interpret the output of their STRUCTURE analyses (Pritchard *et al.* 2000), because the accuracy of STRUCTURE is affected by IBD (Meirmans 2012), and by uneven sampling (Puechmaille 2016).

In analyses that did not consider populations, such as observed heterozygosity for each locus across populations (Table III in Kheng *et al.* 2018), genetic polymorphism using SNPs, and nucleotide diversity in noncoding regions (their Table IV), the findings as reported by Kheng *et al.* (2018) are unaffected.

Phylogeography and Isolation by Distance

With a better understanding of the origin of most samples, we can calculate the geographic distance between any two samples and relate that to their genetic distance. We used a Generalized Additive Model (*gamlss* package in R) and selected a power exponential distribution to describe the IBD relationship based on the AIC ($R^2 = 0.19$, $\Delta AIC = 114$ in relation to the null model; estimate: 0.03, SEM = 0.003, t -value: 7.89, $P < 0.0001$). There is a strong correlation between genetic distance in the mtDNA control region between two gibbons and the straight-line geographic distance between the locations where they were sampled (Fig. 1). IBD is a simple consequence of limited dispersal and individuals (and populations) that are geographically close tend to be genetically more similar than individuals (and populations) that are far apart. Sex-biased dispersal may result in different levels of genetic structure in markers with different inheritance patterns (e.g., mtDNA vs. nDNA) and the sex that disperses less tends to present a stronger overall

genetic structure. In gibbons, males and females disperse over similar distances (typically one, two, or three home ranges), making the choice of markers to tests IBD less important than in species with sex-biased dispersal. Under drift and short-range gene flow, genetic similarity for neutral loci declines with geographic distance, and IBD can be easy to conflate with population structure (Meirmans 2012). Thus, genetic similarities between close populations do not necessarily reflect selection.

Implications for the Conservation Genetics of Javan Gibbons

With respect to the conservation and management of Javan gibbons, the most encouraging aspect of the Kheng *et al.* (2018) study is that levels of genetic variation in mtDNA and nDNA are comparable to those of other gibbons, and that there is no indication of a substantial loss of nDNA variation in the species as a whole. A model of the likelihood of survival of three large Javan gibbon populations (Ujung Kulon, Mts. Halimun–Salak, and Mts. Dieng) found that inbreeding depression (3.14 lethal equivalents per diploid genome on juvenile mortality) had no measurable effect on extinction risk in the medium–long term (100 years) (Smith *et al.* 2017). Supriatna *et al.* (1999) and Andayani *et al.* (2001, p. 774) noted that “releasing confiscated gibbons ... into the wild population on Java should be avoided except under the most stringent conditions of taxonomic identification.” However, Javan gibbons have been released in at least five areas in recent years, viz. Takokak, Mt. Tilu, Mt. Malabar, Mt. Gede, and Mt. Pangrango. These released gibbons comprise a mixture of confiscated wild-caught, captive-born, and captive-bred individuals predominantly from Java, but also from zoos outside Indonesia. As far as we are aware, no genetic tests have been conducted to assess the geographic origin, or the putative ESU, of these released individuals or their parents. While this may augment gene flow, in the absence of any evidence of inbreeding depression in Javan gibbons, it is doubtful whether or not populations would benefit from this.

Given our alternative interpretation of the data presented by Kheng *et al.* (2018), there is currently not enough support to justify an ESU-level management strategy for conservation practices. Increased sampling of known localities would provide clearer evidence for/against the existence of ESUs and their potential role in conservation management of the Javan gibbon.

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