



Research

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Population genetics

Recent divergences and size decreases of eastern gorilla populations

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Compared with other African apes, eastern gorillas (*Gorilla beringei*) have been little studied genetically. We used analysis of autosomal DNA genotypes obtained from non-invasively collected faecal samples to estimate the evolutionary histories of the two extant mountain gorilla populations and the closely related eastern lowland gorillas. Our results suggest that eastern lowland gorillas and mountain gorillas split beginning some 10 000 years ago, followed 5000 years ago by the split of the two mountain gorilla populations of Bwindi Impenetrable National Park and the Virungas Massif. All three populations have decreased in effective population size, with particularly substantial 10-fold decreases for the mountain gorillas. These dynamics probably reflect responses to habitat changes resulting from climate fluctuations over the past 20 000 years as well as increasing human influence in this densely populated region in the last several thousand years.

1. Introduction

Among the African apes comprising our closest living relatives, the eastern gorillas (*Gorilla beringei*) are particularly intriguing but relatively little studied. The few samples analysed to date possess strikingly low genetic variation as compared with humans and other apes, including the closely related western gorillas [1–3]. Genetic evidence suggests that the ancestral western and eastern gorilla populations split some 1 Ma, but that gene flow persisted until as recently as 200 000 years ago [1,2].

The evolutionary relationships among the eastern gorillas themselves are poorly understood. Patchily distributed primarily in the eastern Democratic Republic of Congo, the little-studied eastern lowland gorillas (*Gorilla beringei graueri*; Grauer's gorilla) number perhaps in the tens of thousands (figure 1). Approximately 480 mountain gorillas of the Virunga Massif population live at altitudes above 2500 m and are separated by only 30 km from the approximately 400 mountain gorillas living at 1400–2500 m in the Bwindi Impenetrable Forest [4]. Recent migration between the two populations is unlikely as both gorilla populations are surrounded by a very high density of humans (approx. 300 people km⁻²). Although dietary differences arising from ecological differences are evident, morphological differences between the two mountain gorilla populations are slight and both mountain gorilla populations are classified as the same species [2]. It has been suggested that the two mountain gorilla populations split only 500 years ago following a major increase in human population density and the clearance of forest between both habitats [5].

Genetic inferences of the evolutionary histories of eastern lowland and mountain gorilla populations have exclusively relied upon mitochondrial DNA (mtDNA) sequence analysis. The mtDNA evidence suggests a molecular divergence between eastern lowland gorillas and mountain gorillas (*G. b. beringei*)

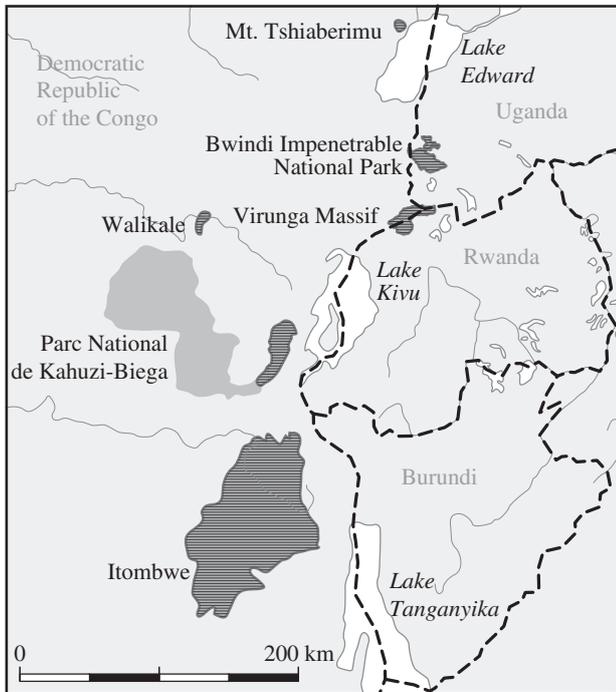


Figure 1. Darkly shaded areas indicate the sampled eastern gorilla populations.

around 400 000 years ago, but the population split may well have been more recent [6].

Here we use data from a set of rapidly evolving, neutral autosomal microsatellite loci characterized in a total of 150 eastern gorillas non-invasively sampled in the wild to examine the evolutionary histories of eastern lowland and mountain gorillas using Bayesian coalescence-based methodologies [7,8]. Our aims are to estimate the time at which the ancestral eastern lowland gorilla population split from the ancestral mountain gorilla population, as well as the time of the subsequent split between the two mountain gorilla populations, and to infer current and past effective population sizes (N_e s). Elucidating past population dynamics in eastern gorilla populations will allow us to set our results within the broader context of climatological or anthropogenic factors that may have influenced the evolutionary history of eastern gorillas and other African mammals.

2. Material and methods

Using DNA extracted from faecal samples collected non-invasively in the course of mountain gorilla surveys in the Virunga Massif and Bwindi Impenetrable National Park, we generated autosomal microsatellite genotypes from a total of 255 and 195 individuals from the Virungas and Bwindi, respectively, as previously described [4]. We similarly analysed 64 Eastern lowland gorilla samples from various localities (electronic supplementary material; figure 1). For the analyses reported here, we used 50 randomly selected genotypes from each of the three populations and compared nine loci between mountain gorilla populations and eight loci between eastern lowland and mountain gorilla populations (electronic supplementary material, table S1).

The software *IMA2* [8] was applied to the population pairs (i) Virunga–Bwindi, (ii) Virunga–Eastern lowland, and (iii) Bwindi–Eastern lowland to obtain the posterior estimate of the divergence time (t_0) since populations (or subspecies) split from a common ancestor, as well as the ancestral and current effective population sizes (N_e). We did not consider all

three populations (Virunga, Bwindi and Eastern lowland) in the same model, but rather conducted pairwise analyses as recommended when limited data are available [8].

We used *MSVAR* 1.3 [7] to assess the magnitude and the timing of the most recent major demographic change in the mountain gorillas. Based on lognormal prior and hyperprior distributions whose means and standard deviations are defined by the user, this method estimates four natural (i.e. unscaled) parameters: (i) the current effective population size (N_0), (ii) the initial effective population size (N_1), (iii) the mutation rate (μ), and (iv) the time (x_a) since the population started to decline/expand. For each population, we applied both the linear and the exponential models and ran seven independent simulations with different starting parameter values and random seeds for each population (electronic supplementary material, tables S2 and S3). Further details on the *IMA2* and *MSVAR* analyses are provided in electronic supplementary material, figure S1.

3. Results

Using three pairwise population analyses of isolation with migration (*IMA2*), we estimated that the Virunga and Bwindi mountain gorilla populations each split from the eastern lowland population approximately 10 000 years ago (table 1), while the split between the two mountain gorilla populations started around 5000 years ago. These estimates suggest that the Virunga and Bwindi mountain gorilla populations would have been in reproductive contact for approximately 5000 years after the onset of the split from the ancestral eastern lowland population. Our data did not support estimation of migration parameters in the models and hence we cannot gauge the magnitude or duration of any continued migration between gorilla populations after the onset of the divergence events.

The *IMA2* analyses also suggested larger ancestral effective population sizes (N_e s) as compared with current effective population sizes for all three eastern gorilla populations (table 1). For example, at 4244, the N_e of the ancestral mountain gorilla population is several-fold larger than the 352–450 estimated for either present-day mountain gorilla population. Furthermore, there is no overlap between 95% highest posterior density (HPD) intervals for ancestral and present-day mountain gorilla N_e s (table 1), suggesting drastic population declines following the onset of the divergence of these populations. The results hint at a less drastic decline in the N_e of the eastern lowland population, with the point estimate reduced by approximately twofold to a current-day N_e of around 1500, but with overlapping 95% HPD intervals. Estimates of N_e are similar for any given population across models, with current N_e estimates of around 352–450 for the mountain gorilla populations, approximately one third of the 1385–1520 estimated for the current eastern lowland gorilla population.

Given these results suggesting a substantial decline in mountain gorilla effective population sizes, we also used *MSVAR* 1.3 to estimate the magnitude and the timing of the most recent major change in the effective size of both mountain gorilla populations. We found that the highest posterior value of the initial effective size (N_1) of either mountain gorilla population at about 2500–3400 is approximately 10–12 times larger than the current sizes (N_0) of 200–300 (table 2). The estimates of the time since the populations started to decline are, for each population, twice as deep for the linear model as for the exponential model and are approximately 5000 or 10 000 years ago for the Virunga population

Table 1. Highest posterior probability values and, in brackets, 95% HPD intervals, for the parameters estimated using *ima2*. Results are listed for each of the six independent runs performed on each pairwise population analysis. Values in bold are the arithmetic means (\pm s.d.) calculated over the six runs. N_e denotes the effective size of a population and 'Eastern' indicates the eastern lowland gorilla population.

pairwise analysis	divergence time (t_0) in years	current N_e , pop1	current N_e , pop2	ancestral N_e
Virunga (pop1)–Bwindi (pop2)	4740 [1260–13 980]	369 [131–681]	456 [169–831]	4419 [2169–8544]
	5580 [1860–13 980]	369 [156–669]	481 [206–831]	4319 [2156–8506]
	4980 [1500–13 980]	344 [131–656]	406 [181–831]	4169 [2144–8356]
	5220 [1740–13 860]	344 [156–681]	456 [194–819]	4094 [2094–8331]
	6060 [1860–13 980]	356 [156–669]	431 [194–844]	4206 [2119–8594]
	4980 [1740–14 580]	331 [144–681]	469 [194–844]	4256 [2131–8356]
	5260 (\pm 484)	352 (\pm 15)	450 (\pm 27)	4244 (\pm 115)
Virunga (pop1)–Eastern (pop2)	9420 [2460–89 580]	499 [219–919]	1496 [761–2861]	3386 [884–7481]
	11 220 [3300–96 540]	551 [254–954]	1601 [814–2861]	3351 [761–7866]
	10 980 [2820–119 820]	551 [236–954]	1584 [761–2984]	3351 [359–7149]
	10 980 [2580–118 260]	534 [236–919]	1496 [726–2844]	3404 [971–7481]
	11 460 [2700–112 020]	516 [236–936]	1496 [726–2826]	3386 [779–7919]
	11 340 [2940–119 940]	551 [236–936]	1444 [779–2861]	3456 [604–7481]
	10 900 (\pm 750)	534 (\pm 22)	1520 (\pm 60)	3389 (\pm 39)
Bwindi (pop1)–Eastern (pop2)	9540 [1980–25 860]	551 [254–1041]	1461 [621–2669]	4244 [2091–8479]
	8580 [2580–23 820]	569 [271–1024]	1444 [516–2599]	4279 [2021–8339]
	8700 [2340–24 540]	604 [236–1024]	1286 [586–2581]	4349 [2074–8689]
	8100 [2220–28 020]	604 [254–1059]	1339 [621–2739]	4261 [1969–8636]
	9420 [3300–27 300]	551 [289–1059]	1461 [709–2651]	4209 [2021–8111]
	9300 [2340–25 020]	604 [254–1041]	1321 [551–2651]	4366 [2021–8321]
	8940 (\pm 568)	580 (\pm 26)	1385 (\pm 79)	4285 (\pm 61)

Table 2. Highest posterior probability values and, in parentheses, 95% HPD intervals for three parameters estimated using *MSVAR* 1.3. for the two mountain gorilla populations. N_e denotes the effective size of a population.

population	model	current N_e (N_0)	initial N_e (N_1)	time since decline (X_a)
Virunga	linear	187 (2–3726)	2486 (219–34 518)	9987 (97–1 685 280)
	exponential	240 (4–3953)	2525 (215–30 654)	5216 (12–362 356)
Bwindi	linear	289 (8–4707)	3334 (280–46 793)	20 411 (378–1 610 498)
	exponential	334 (17–4302)	3357 (307–40 268)	10 336 (159–387 194)

and 10 000 or 20 000 years ago for the Bwindi population (table 2). Our results suggest that the onset of the decrease in effective population size may have been earlier for the Bwindi population as compared with the Virunga population, or if one assumes the declines occurred at the same time, the magnitude of the decline would have been more intense in Bwindi (table 2).

4. Discussion

Our analyses suggest rather recent population splits among eastern gorilla populations, with an initial split of the ancestral eastern lowland and mountain gorilla populations commencing about 10 000 years ago and a subsequent split of the Virunga and Bwindi mountain gorilla populations some 5000

years ago. These values are compatible with studies of the more distant evolutionary relationship between the western and eastern gorilla species, which find deeper split times of 0.9–1.6 Ma [1] and 1.75 Ma [2], but with a cessation of gene flow only around 80 000–200 000 years ago [1]. Our split time estimate for eastern lowland and mountain gorillas is much more recent than the mtDNA divergence estimate of 316 000–443 000 years ago [6], which reflects the difficulties of deriving population split times from the molecular divergence of a single locus such as mtDNA, particularly for species that may experience predominantly male long-distance gene flow [1]. Unfortunately, although our eight biparentally inherited and independent genetic markers lead to more informative split times than does mtDNA, the data do not suffice to estimate the extent, duration or direction of any migration between diverging eastern gorilla populations.

The presumed cause of splits between gorilla populations is changes in forest distribution. The 95% HPD intervals associated with our estimates of the split times between either of the two mountain gorilla populations and the eastern lowland population encompass the time of the last world glacial maximum that peaked around 18 000 years ago and caused forest reduction due to increased aridity [9]. Pollen data showing persistence of forest canopy at or near Bwindi suggest that the loss of vegetation types during the last glacial maximum was significantly less pronounced at high-altitude sites compared with the lower-altitude lowland forest habitat, causing the forest-dependent gorilla to shift its range upward during the last arid period. An alternative scenario might be that the split between the ancestral mountain gorilla and eastern lowland populations followed the recolonization of high-altitude forest environment following the moist forest expansion towards the end of the Pleistocene some 12 000–9000 years ago.

Our analyses also highlight the strong decline in the mountain gorilla effective population sizes over the past 5000–10 000 years, subsequent to their split from eastern lowland gorillas. Both climatic and anthropogenic factors are known to have influenced the area during the Holocene period, with progressively drier conditions and accompanying vegetation changes in the past 5000 years and evidence of human-induced environmental change only in the past approximately 900 years [10]. In sum, our analyses suggest that the histories of eastern gorilla population reflect habitat changes associated with long-term climate fluctuations and more recent human activities.

Data accessibility. The data used are presented in the electronic supplementary material.

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