Habitat fragmentation and its implications for Endangered chimpanzee *Pan troglodytes* conservation

ALEXANDER KNIGHT, HAZEL M. CHAPMAN and MARIE HALE

Abstract Taraba State, Nigeria, is an important conservation site for the Endangered Nigeria–Cameroon chimpanzee *Pan troglodytes ellioti*. Gashaka Gumti National Park, Nigeria's largest national park and home to potentially the largest contiguous population of the Nigeria–Cameroon chimpanzee, spans a significant portion of the eastern sector of Taraba and the adjoining Adamawa State. South of the Park, Ngel Nyaki Forest Reserve comprises two forest fragments and holds a small population of chimpanzees. We investigated the existence of patterns in population structure and dispersal within this region, using microsatellite loci extracted from non-invasive sources of DNA. Our results indicate that dispersal and thus gene flow between the groups of chimpanzees at the Park and Reserve is limited, at least more so than it is within the Park, and we identified a biased sex ratio at the Reserve, forewarning of potential conservation concerns in relation to demographic and genetic stochasticity. We discuss conservation actions that may be applicable to sustaining the population within Ngel Nyaki Forest Reserve.

Keywords Assignment tests, chimpanzees, conservation, microsatellites, Nigeria, *Pan troglodytes*, population structure

The forest fragments of southern Taraba State, Nigeria, have been recognized as an important conservation site for the Endangered Nigeria–Cameroon chimpanzee *Pan troglodytes ellioti* (Morgan et al., 2011). Chimpanzees are threatened primarily by deforestation and illegal poaching for the bushmeat trade (Kormos et al., 2003; Morgan et al., 2011). Within Taraba State both Gashaka Gumti National Park (6,731 km²) and Ngel Nyaki Forest Reserve (c. 7 km²; Fig. 1) have relatively high levels of protection for the region. Both are patrolled by rangers and have research stations within or on the periphery of their forest. Researchers and assistants conduct work throughout the forests, providing a practical deterrent to poachers, at least in certain areas. Gashaka Gumti is Nigeria's largest national park; in contrast Ngel Nyaki comprises two small forest fragments. The chimpanzee population in Gashaka Gumti is estimated to be c. 1,000 individuals (Morgan et al., 2011). In Ngel Nyaki nest count surveys have estimated the population to be c. 12 individuals (Beck & Chapman, 2008) but observations indicate there may be as many as 16 (Dutton, 2012). Little is known about the ranging behaviour of the Nigeria–Cameroon chimpanzee and whether it varies from that of other subspecies, but population genetic theory dictates that stochastic factors having a negative impact on persistence become increasingly important in determining the viability of diminishing and isolated populations (Frankham et al., 2002). Gashaka Gumti and Ngel Nyaki are bisected by one of the few paved roads in the region (Fig. 1). The villages of Yelwa and Maisamari are located along the section of the road between the Park and the Reserve and are surrounded by agricultural land. We investigated the population structure and patterns in dispersal and gene flow between the Park and the Reserve, using microsatellites extracted from non-invasive sources of DNA.

During January–April 2012 a total of 85 faecal samples were collected in Gashaka Gumti and Ngel Nyaki, of which 57 yielded amplifiable DNA. From these 57 samples we identified 32 unique genotypes, using a multiple tubes procedure (Navidi et al., 1992). The number of amplifications required to confirm a genotype was determined by a probability formula; heterozygotes were amplified at a minimum twice per allele (with at least one visible heterozygote), and homozygotes three times, with the exception of one locus. As a result of higher rates of allelic dropout and false alleles at this locus, heterozygotes were amplified a minimum of three times, and homozygotes four times (Morin et al., 2001). The majority of the samples were typed for 7–8 loci, three samples were typed for six loci, one for five loci, and another for four loci.

We found no evidence of departures from Hardy–Weinburg equilibrium, or of linkage disequilibrium. We tested for isolation by distance using a mantel test and found a correlation between genetic dissimilarity and geographical distance ($r = 0.1051$, $P = 0.016$) when all the samples were included in the analysis. There was no correlation ($r = 0.04314$, $P = 0.216$) when the analysis was

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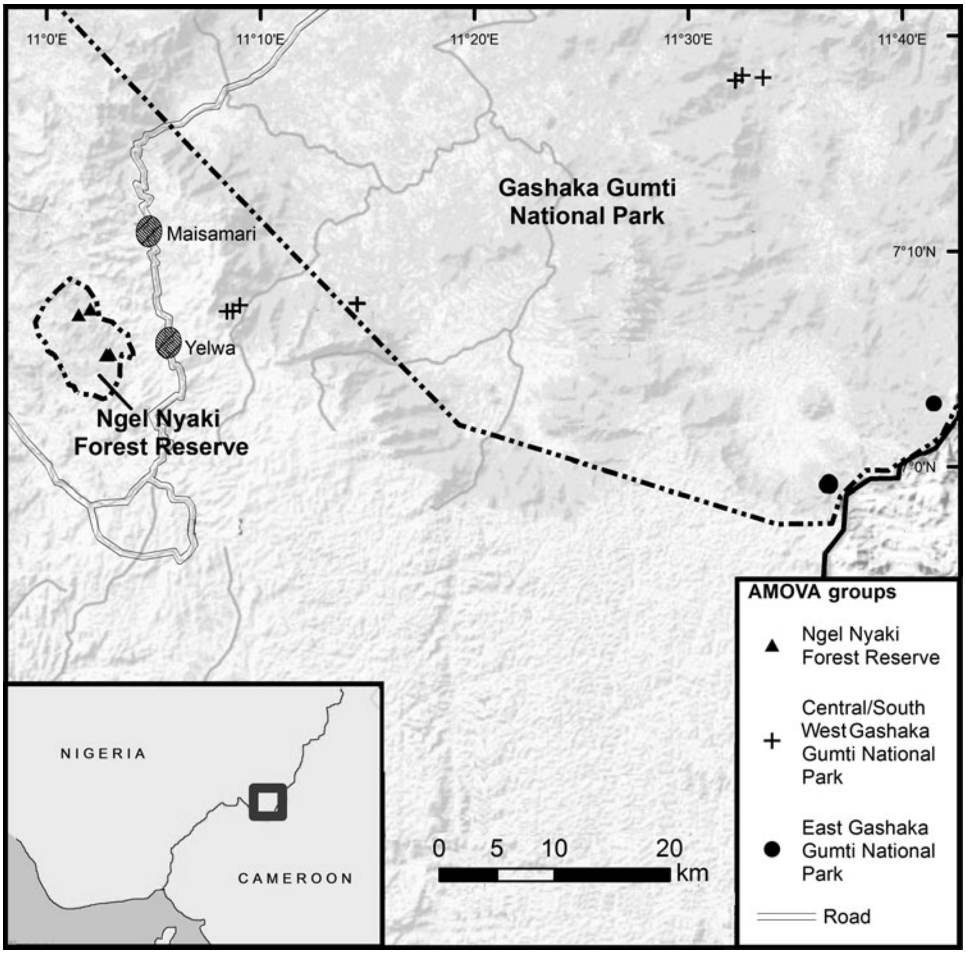

FIG. 1 Sample locations of the Nigeria–Cameroon chimpanzee *Pan troglodytes ellioti* in Ngel Nyaki Forest Reserve and Gashaka Gumti National Park, in Taraba State, Nigeria, symbolized by the groups determined by AMOVA.

restricted to samples collected only in Gashaka Gumti. We investigated potential patterns in population structure using an AMOVA (analysis of molecular variance), which indicated that the samples we collected in Gashaka Gumti may be from two groups of chimpanzees, one located in the eastern sector of the Park and one in the central and south-west ($F_{ST} = 0.027$, $P = 0.018$). The samples from Ngel Nyaki appeared to be from a third distinct group ($F_{ST} = 0.047$, $P = 0.003$ and $F_{ST} = 0.027$, $P = 0.028$, respectively). We used an assignment test to examine dispersal and gene flow among these groups. Assignment tests generate log likelihood values based on allele frequencies for samples belonging to any pre-designated populations. When individuals are misassigned this may indicate dispersal among the pair of populations being tested, or that allele frequencies are not sufficiently different for the test to be able to discern the true population of origin, potentially as a result of gene flow among the populations. When the samples were grouped according to the results of the AMOVA, seven of the 24 (29%) samples collected in Gashaka Gumti were misassigned to either the eastern or central/south-west group (Fig. 2). In contrast, one sample was misassigned from each comparison between Ngel Nyaki and the groups in Gashaka Gumti (5.2%, eastern group, Fig. 2; 4.7%, central/south-west group, Fig. 2).

Our results indicate that the group of samples from Ngel Nyaki represents a genetically distinct population from the group or groups residing in Gashaka Gumti. This may be attributable to the distance between the two regions, or it may be because the lack of forest, land use, and relatively higher human population density act as a barrier to dispersal. Excluding Gashaka Gumti, the nearest chimpanzee population, to the best of our knowledge, is located 30 km south. Whether it is possible for chimpanzees to traverse this distance is unknown. There are unsurveyed regions in the vicinity of Ngel Nyaki that could potentially harbour viable chimpanzee populations (Morgan et al., 2011). The results of the AMOVA suggest there may be at least two genetically distinct groups of chimpanzees within Gashaka Gumti. Chimpanzees are known to form male philopatric communities (Wilson & Wrangham, 2003), and both of these groups would contain multiple males, which could be driving the genetic differentiation. The assignment test, however, misassigned a significant proportion of the individuals in these groups, suggesting frequent dispersal resulting in gene flow. There were comparatively lower frequencies of misassignments in comparisons between Ngel Nyaki and the groups within Gashaka Gumti, suggesting that individuals do not disperse as frequently between these locations, and gene flow is less common than within the Park. As the log likelihood values are based on allele frequencies, the outcome of the test is dependent on sample size. The sample sizes are modest, and therefore the results should be interpreted with caution.

If the population at Ngel Nyaki is isolated from the National Park, conservation measures need to be considered if it is to be sustained into the future. Investigating whether any other populations exist to the south, east or west, and whether there is any evidence of connectivity between these should be a priority. A complete demographic profile of the population at Ngel Nyaki is critical for making the most informed decisions. We identified eight individuals, of which one was male. If this accurately reflects the sex ratio, the population is already at immediate risk of extinction, from the loss of one sex. However, observations indicate that the population may be larger; if so, knowing the sex ratio will help determine several factors, including the probability of persistence and the rate at which genetic diversity may be lost. The mean expected heterozygosity was 0.792, similar to that reported from a sample of 115 chimpanzees in Côte d'Ivoire (0.798, Boesch et al., 2006). The biased sex ratio could result in a rapid reduction in genetic diversity (Frankham et al., 2002), and therefore we recommend continual monitoring of the population at Ngel Nyaki. Given that a female's inter-birth interval is c. 4 years (Wallis, 1997), surveying the genetic diversity in the population should be undertaken every 4–5 years to record new births.

Theory suggests that in a population of this size the negative effects of inbreeding depression are a serious concern, and strategies to combat this should be formulated (Frankham, 2003). Mitigating the accumulation of deleterious alleles requires the introduction of genetic diversity (Frankham, 2005). If it is apparent that there is no connectivity between Ngel Nyaki and any other region, Gashaka Gumti holds the closest population from which this is possible. A wildlife corridor between the two regions could potentially encourage natural gene flow, although mitigating deforestation in the region is already problematic and the distance between the two regions is not trivial. We consider this endeavour to be worthwhile, however, as it would also benefit other species. Translocation of chimpanzees is another potential solution but this has had mixed results and is complicated by the territorial nature of male

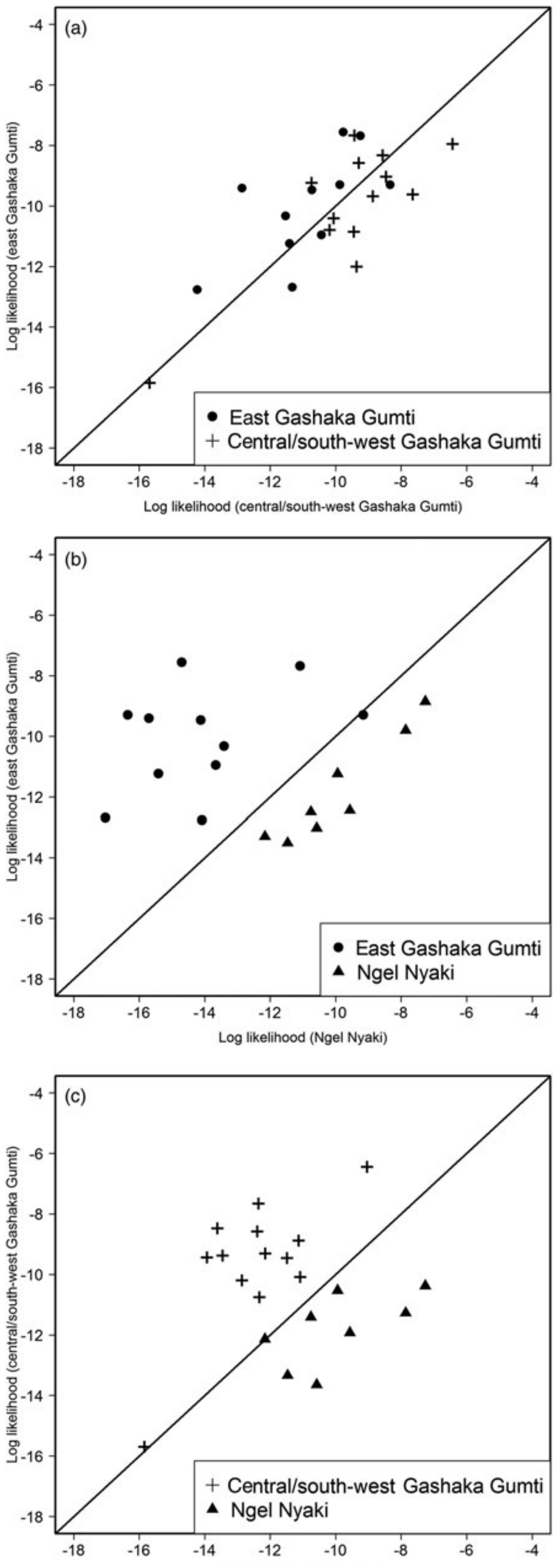

FIG. 2 Log likelihood values for individual chimpanzees being assigned to one of two groups being tested in each pairwise combination of groups as determined by AMOVA. The groups tested are (a) east Gashaka Gumti and central/south-west Gashaka Gumti, (b) east Gashaka Gumti and Ngel Nyaki, and (c) central/south-west Gashaka Gumti and Ngel Nyaki. The diagonal line in each plot represents the point at which log likelihood values are equal and an individual may be assigned to either population.

chimpanzees (Goossens et al., 2003, 2005). If there is only one male at Ngel Nyaki and this male dies then attempting male translocation should be considered, but as long as surveys and observations indicate that there are males present this is not a viable solution. Considering that the level of genetic diversity is not dissimilar to that of a larger population, the risk of introducing pathogens, and the number of females present at Ngel Nyaki, translocation of a female to introduce genetic diversity does not seem warranted at this stage. We recommend monitoring of the population at regular intervals, and discussion regarding the feasibility of constructing a wildlife corridor. The Reserve has been designated a Centre for Technical Forest Science, and the presence of a well-established research station makes it a suitable location to observe and study this rare and elusive subspecies.

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Biographical sketches

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