

# **The effects of landscape features on the distribution and genetic structure of forest duikers (Cephalophinae) in the tropical forest of Moukalaba, Gabon**

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## **Introduction**

African forests are well known for high diversity of mammals and duikers contribute to species richness in mammals. In central and western Africa, several duiker species (subfamily Cephalophinae) live sympatrically. The study of the effects of landscape features on distribution and dispersal is important to understand population structure and distribution patterns of coexistent duiker species in the same forest. However, duikers are difficult to observe directly. In this study, I conducted dung counts with genetic species identification of duikers across several types of habitats and assessed the effect of various environmental factors, including human pressure, on the relative abundance and genetic structure of duiker species.

## **Materials and Methods**

The study was conducted in the northeastern part of the Moukalaba-Doudou National Park which comprised of several vegetation types including old secondary forests, young secondary forests, and swamp forests. Moukalaba also varied in elevation and most of the study area was logged from the 1960s to the 1980s. I conducted the reconnaissance survey and collected fresh piles of ungulate faeces in thirteen 2-km line transects established in various environments. I conducted genetic species identification using mitochondrial control region. I examined the effect of

environmental factors on the dung abundance of each duiker species using generalized linear mixed model. Molecular techniques could not discriminate between Ogilby's (*Cephalophus ogilbyi*) and Peter's (*C. callipygus*). I considered those species as Ogilby's duiker and the data were not used in population genetic structure analyses. To assess the population genetic structure in each duiker species, I developed original nine microsatellite markers for duikers using next-generation sequencing technology. Population differentiation was analyzed using eleven microsatellite loci, including the markers developed for other bovine species, and mitochondrial D-loop sequences (~600 bp).

## Results

Genetic species identification showed that 146, 83, 35, and 6 faecal samples belonged to blue (*Philantomba monticola*), Ogilby's (*C. ogilbyi*), yellow-backed (*C. silvicultor*), and bay duiker (*C. dorsalis*), respectively. The dung abundance of bay duiker was low compared to other duiker species. Dung of other three species were found in all the transects, but their relative abundance was variable. The dung abundance of Ogilby's duikers was low in young secondary forests and that of yellow-backed duikers was high in swamp forests. In contrast, the dung abundance of blue duiker was not affected by vegetation type and was lower in transects nearer to the villages and in transect with steeper topology.

Individual identification analyses using microsatellite genotypes showed that 22% of samples were duplicate samples. In bay duiker, there were two different haplotypes of mitochondrial DNA. Median-joining network analyses of mitochondria D-loop revealed that there were three and four haplogroups in yellow-backed and blue duiker, respectively. Individual-based Bayesian clustering analysis of blue and yellow-backed duiker showed two

clusters in each species. The results of mitochondrial and microsatellite showed that there were two genetic groups which showed clear geographic separation.

## **Discussion and conclusion**

In this study, genetic species identification showed that four duiker species coexisted. Genetic analyses successfully identified Ogilby's and bay duiker, which were difficult to discriminate using other methods and we found low abundance of bay duikers. This result suggests the importance of accumulating information regarding the abundance of each duiker species for updating their conservation status.

Landscape features which affected the abundance of duikers were different among species. In particular, the dung abundance of Ogilby's duikers and blue duikers was affected by human impacts, while that of yellow-backed duikers was not affected. Habitat disturbance was suggested to affect the distribution of Ogilby's duikers, and that of blue duiker was suggested to be affected by hunting activity. Human impacts may be one of the important factors for determining the abundance of duikers.

Genetic structure analyses suggested that there exist two subpopulations in all the three species. It was difficult to expect the genetic barriers of bay duiker because of small sample size. Mountain was suggested to be responsible for observed genetic differentiation in blue duiker, although a little gene flow was observed between subpopulations. Considering that the dung abundance of blue duikers was low in mountain area, utilization and movement in mountains may be limited in blue duikers. In contrast, river might act as a genetic barrier in yellow-backed duiker. Predation risk of yellow-backed duiker, the largest species, around the river may be high, which makes yellow-backed duikers to avoid taking a risk of crossing the river.

In conclusion, noninvasive genetic method provided new information on duiker species which were difficult to observe directly. Using this technique, I found that several duiker species coexist in various environment and the effects of landscape on distribution and dispersal were different among them. Further ecological studies will clarify the details of their coexistence.