Enhancing knowledge of the ecology of a highly elusive species, the okapi (*Okapia johnstoni*), using non-invasive genetic techniques

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ABSTRACT

Okapi (*Okapia johnstoni*) are an even-toed ungulate in the family Giraffidae, and are endemic to the Democratic Republic of Congo (DRC). Very little is known about okapi ecology in the wild. We used non-invasive genetic methods to examine the social structure, mating system and dispersal for a population of

okapi in the Réserve de Faune à Okapis, DRC. Okapi individuals appear to be solitary, although there was some evidence of genetically similar individuals being associated at a very small spatial scale. There was no evidence for any close spatial association between groups of related or unrelated okapi but we did find evidence for male-biased dispersal. Okapi are genetically polygamous or promiscuous, and are also likely to be socially polygamous or promiscuous. An isolation by distance pattern of genetic similarity was present, but appears to be operating at just below the spatial scale of the area investigated in the present study. We therefore here provide new ecological information about a species that has recently been recognised by the IUCN as Endangered, and is a potentially important flagship species for Central Africa.

INTRODUCTION

The key to protecting and managing species of conservation concern is a good understanding of their ecology, including knowledge of their dispersal, sociality and mating system [1,2]. This information can have a considerable and very real impact on conservation (e.g. [3-5]). However, measuring these factors in wild animals by direct observation can often be very difficult, especially for elusive mammals, or those inhabiting difficult terrain [6,7].

There is a vast amount of variation in social structure, mating systems and dispersal strategy amongst mammals, even among those that are taxonomically and geographically similar [8-14]. This variation in social structure makes predictions of ecological and genetic ???(processes) difficult for any poorly studied mammal species. In terms of social structure, mammals that utilise

densely forested habitats tend towards forming a smaller social unit, putatively because the coordination of a social group is difficult in a forest especially if the animal is large [15]. Also, animals at greater risk of predation are more likely to adopt a hiding strategy [15] and be predominantly solitary to reduce social interaction and therefore detection probability [16].

Mating systems are even more diverse (20) and difficult to predict. For example, the extent of polygamy can be affected by predation pressure [17], social group composition [18] and phylogeny [19]. Due to this complex interaction, mammals show a diverse array of mating systems, true for both males and females [20]. Dispersal (specifically natal dispersal [21]) also often varies between sexes, with some degree of sex-biased dispersal being virtually ubiquitous in mammals [22]. However, male-biased dispersal is the norm for mammals [23]. Due to this lack of predictive power of habitat and taxonomy, other methods are clearly needed to accurately elucidate the ecology of elusive, or otherwise difficult to observe animals.

Non-invasive genetic methods are increasingly being used to investigate questions such as dispersal, mating systems and social structure in wild animals [24-26]. These methods may therefore provide a means of investigating the ecology of elusive animals without actually observing them. The okapi is a highly elusive even-toed ungulate, endemic to the Democratic Republic of Congo (DRC). Although widely distributed throughout the DRC, it occurs at low density across its range [27]. Okapi appear to only be present in dense forest, away from human presence [28,29]. Determining aspects of behavioural ecology using observations

is therefore difficult for this species. Only two *in situ* ecological studies of okapi have been published [30,31]. However the studies are somewhat equivocal, are lacking in detail, and tell us nothing of okapi mating systems or dispersal. Non-invasive genetic methods therefore potentially provide a useful tool for the study of this species.

We hypothesised that okapi are mostly solitary, due to their utilisation of dense rainforest, and the likelihood of them having a high predation pressure [30]). In captivity, okapi males are rotated among females and sire multiple offspring [32]. We hypothesised that this would also be true in the wild, with okapi showing evidence of genetic polygamy, or promiscuity. We also hypothesised that okapi would demonstrate male-biased dispersal, due to its higher incidence in mammals. The above hypotheses will be tested using dung samples from okapi in a population in the okapi faunal reserve (Réserve de Faune à Okapis, RFO), DRC.

METHODS

Study species and site

Okapi are an even-toed ungulate in the family Giraffidae, separated from the giraffe by an estimated ~16 million years of independent evolution [33]. The limited number of long-term ecological studies that have been carried out on okapi have been based in the RFO [30,31] and this reserve was also chosen for the present study (Figure 1). Four teams sampled the park, between December 2010 and February 2011, and collected 208 putative okapi fecal samples. These samples were collected as part of a great ape and human monitoring survey [34],..Briefly, surveys comprised a total of 164X one km transects, and fecal

samples were collected on and between transects [34]. Transect location was determined randomly using the program DISTANCE 6.0 [35]. Each transect was walked once.

DNA extraction and amplification

DNA was extracted from faecal samples (stored in 100% ethanol for 24 hrs and then silica) using a QIAamp DNA Stool Mini Kit (Qiagen). Thirteen microsatellite loci were amplified using the primers Oka-01–13 and PCR conditions from Stanton et al. [36]. Primers Oka-02, 10 & 11 were excluded from the analysis due to low PCR amplification success rate. From the 208 faecal samples, consensus genotypes were generated for 105. These 105 samples were confirmed to be okapi based on the following: 1) Correct species identification from this survey was 100% based on mitochondrial DNA analysis of a subset of samples (Stanton et al (submitted)). 2) Genetic structure and distance analysis of microsatellite data in the present study did not identify any unusually different genotypes within the 105 genotyped samples.

The primer sequences SRY 1 (5' CTTCATTGTGTGGTCTCGTG 3') and SRY 2 (5' CGGGTATTTGTCTCGGTGTA 3'; Wilson and White [1998]) were used to amplify a fragment in 5 blood samples from captive male okapi. Internal primers OJSEX-F (5' CGTGAACGAAGACGAAAG 3') and OJSEX-R (5' TCAATATCTGTAAGCCTTTTCC 3') were designed to amplify a shorter 101 bp fragment in non-invasive okapi samples. Sexing primers were multiplexed with an internal control, Oka-01 (forward: 5' AAGAGAGACTGCACTGTGGACC 3', reverse: 5' GCTCTTGTGTCTGACATGTTCTC 3', [36]). PCR was carried out in a 6.5 µl volume

with 2.5 µl Multiplex Mix (Qiagen), 4 µg BSA, 2 nmol OJSEX primer, 0.8 nmol Oka-01 and 2 µl DNA. The PCR was carried out twice for each of the samples that had been successfully genotyped, always with two negative controls. A sample was accepted as a female if both reactions showed the absence of a band from the sexing primers.

Primers Mt 1 – 5 (Stanton et al. (submitted)) were used to amplify a fragment of the mitochondrial DNA control region (mtDNA CR), and cytochrome b, tRNA-Thr and tRNA-Pro genes in individuals with sexing information, using the conditions from (Stanton et al. (submitted)). A 325 bp fragment was amplified in 20 individuals (females n = 9, males n = 11), and a 543 bp fragment (that included the 325 bp fragment above) was amplified in a further 15 individuals.

Data validation

A preliminary genotyping error rate study was carried out using the programs PEDANT [38] and GEMINI [39] on 14 okapi faecal samples, comparing two genotyping repeats of each sample. GEMINI indicated that 2-3 repeats would be required to be able to accept a consensus genotype with >95% confidence, and PEDANT calculated an allelic dropout rate for each locus at between 0.0170 and 0.1645 (mean 0.0779), a false allele rate of between 0 and 0.0718 (mean 0.0170). The confidence converged on 100% with approximately three repeats. Therefore, for caution, at least four repeats (and up to eight) for each of the samples in the full study were carried out. Genotyping error rates were then recalculated on the full dataset. The allelic dropout rate for each locus was between 0 and 0.0429 (mean 0.0161) and false allele rate was between 0 and

0.0055 (mean 0.0010), demonstrating that the four repeats carried out were sufficient to give reliable consensus genotypes at the 95% level.

Spatial autocorrelation

To test the hypothesis of low social structure in okapi, the relationship between proximity of okapi dung samples and genetic distance was investigated. This was to determine if related individuals are spatially more closely associated than unrelated individuals, and was carried out using spatial autocorrelation analysis (SAA). Spatial autocorrelation measures the degree of dependency of observations, for example genetic distance, across space. Significant positive autocorrelation (in the example of genetic distance) indicates that genetically similar individuals are closer together than one would expect by chance, whereas significant negative autocorrelation indicates that individuals are arranged to maximize genetic distance between them [40]. We carried out spatial autocorrelation analysis using GenAlEx v6.4 [41,42], with significance assessed using 95% confidence interval and 9999 permutations. SAA was carried out on males (n = 27) and females (n = 29) separately, and on the combined dataset (n = 83) at distance intervals of (i) 2 km across 20 km, and (ii) 10 km across 120 km. The analysis was carried out on the combined dataset only (n = 83; there was insufficient data to analyse males and females separately) at distance intervals of 0.2 km across 2 km.

Patterns of relatedness

To further describe sociality of okapi in the study site, and to complement the spatial autocorrelation analysis, the association between spatial proximity and

genetic relatedness was investigated. Pairwise relatedness was estimated using the program COANCESTRY v1.0.1.2 [43], which implements seven methods for estimating pairwise relatedness from individual multilocus genotypes. Duplicate genotypes were removed from the dataset and the spatial proximity of related dyads in the remaining individuals (n = 83) was described. This was done by investigating if there were significant differences between average spatial proximity of dyads with a relatedness greater than 0.5 verses less than 0.5, and greater than 0.25 verses less than 0.25, using t-test tests in R (R Development Core Team). This was carried out for all seven estimators. A rarefaction analysis was also carried out on the microsatellite genotypes using the program RERAT [44] to investigate the ability of the 10 markers used in the present study for inferring relatedness.

Multiple dung piles

Eight multiple dung piles (greater than one dung pile ≤ 2 m apart) were found in the study site. Duplicate genotypes were identified, and genetic relatedness was described for these samples, to investigate if these multiple dung piles represent social groups, or single individuals. Multilocus genotypes different at most at only one locus (to account for genotyping errors) were regarded as from a single individual.

Mating system

To investigate the mating system of okapi the relative numbers of half verses full siblings were estimated using the program COLONY [45]. COLONY considers the the two-generation full-pedigree of all sampled individuals, and assigns sibship

and parentage jointly. As the method implemented in this study is effectively using offspring genotypes at autosomal loci, it is unable to determine the polygamous sex. When few half siblings are detected in the COLONY analysis, the mating system is inferred as monogamous for both sexes. Otherwise, it is inferred that either males, females, or both are polygamous. No prior was used for average sibship size, and the defaults for other parameters were accepted in the analysis.

Duplicate genotypes

A direct measure of movement was estimated using identical genotypes, identified in the dataset as dyads with zero or one allele different. Distance between identical dyads was measured, and classified as a natal dispersal event if the distance was greater than the current maximum recorded okapi home-range size (females: 5.1 km^2, males: 10.5 km^2; [30]). All identical dyads less than this distance were classed as 'movement' events.

Spatial genetic structuring

To detect any hidden genetic structure and barriers to okapi movement/dispersal in the reserve, we carried out a Bayesian clustering analysis, and tested for isolation by distance and spatial autocorrelation. Bayesian clustering analysis was performed using the program STRUCTURE 2.3.4 [46], with 500,000 MCMC iterations, a burn-in of 50,000, correlated allele frequencies and K set at 1-5. Isolation by distance analysis was carried out in R (R Development Core Team) using a mantel test to assess the correlation between geographic distance and genetic distance, calculated using GenAlEx

[41,42]. Spatial autocorrelation analysis was also carried out in GenAlEx, using the methods described above.

Sex-biased dispersal

Sex-biased dispersal can be detected by a differences in mitochondrial haplotype diversity [48,49], mAIc and vAIc [22,50], F_{ST} values [51], relatedness estimates [52] and genetic structure [26,53] between males and females. In all sex-biased dispersal analyses, only individuals that had been assigned as either male (n = 27) or female (n = 29), after duplicate genotypes had been removed, were used. Populations for the F_{ST} analysis were the northern half of the RFO verses the southern half, and the western half of the RFO verses the eastern half, with F_{ST} calculated separately for males and females. Pairwise relatedness (Queller and Goodnight method [52]) was calculated for all individuals in the dataset described above (n = 56), using GenAlEx [41,42]. Significant differences were then tested between males and females in R (R Development Core Team) using a t-test. Normality was confirmed visually using histograms and qq plots. Haplotype diversity was calculated in i) all 35 individuals for the 325 bp fragment, and ii) the 15 individuals for which 543 bp of sequence data was available for, using DNAsp v5 [54]. Bayesian clustering analysis was performed using the program STRUCTURE 2.3.4 [46], and the settings described above, separately for males and females to investigate if any differences in dispersal can be detected in differences in genetic structure. FSTAT v2.9.3.2 [50] was used to investigate if there were differences in vAIc and mAIc for males and females in the dataset. A one-sided test was run with 10,000 permutations. Assumptions of the program are that dispersal occurs at the juvenile stage, before reproduction,

and that individuals are sampled post-dispersal. This first assumption is reasonable, however it cannot be determined if our dataset contained pre-dispersal individuals. The power of these statistical descriptors may therefore be lower than expected.

RESULTS

Spatial autocorrelation

Using the 2 - 20 km distance category, we found consistent positive autocorrelations at 4 km ($p < 0.05$) for males, females and the combined dataset. There was also negative autocorrelations in males and females at 14 km and 18 km respectively. When considering the 10 - 120 km distance category: There was a negative autocorrelation at 20 km ($p < 0.05$), 110 km ($p < 0.05$) 80 km ($p < 0.01$) in the female, male and combined datasets respectively. Unexpectedly there was also a *positive* autocorrelation at 50 km ($p < 0.05$) for the male dataset. When considering the 0 – 2 km distance category: There was a positive autocorrelation at 0.2 km ($p < 0.01$) and 1 km ($p < 0.05$), and a negative autocorrelation 0.6 km ($p < 0.05$). When considering the 2 – 20 km distance category: There was a positive autocorrelation at 4 km for males, females and the combined dataset ($p < 0.05$ in all cases). There was also a negative autocorrelation at 14 km for males, and 18 km for females ($p < 0.05$ in both cases). Spatial autocorrelation graphs are shown in Figures 2-4 (males and females combined), Figures 5 & 6 (males only) and Figures 7 & 8 (females only).

Patterns of relatedness

For all seven estimators using COANCESTRY, geographic distance was lower for dyads with a relatedness value greater than 0.5. This difference was significant using some estimators, but not others (LREst: 45.5 km vs 50.2 km, t = 0.816, p = 0.425; TrioEst: 44.7 km vs 48.8 km, t = 1.165, p = 0.250; WEst: 39.3 km vs 48.7 km, t = 1.865, p = 0.826; REst: 47.5 vs 48.7, t = 0.138, p = 0.893; MEst: 42.5 km vs 48.9 km, t = 2.126, p = 0.037; LLEst: 38.8 km vs 48.8 km, t = 2.236, p = 0.038; QGEst: 40.4 km vs 48.7 km, t = 1.17, p = 0.264). There was no significant difference in average geographic distance between dyads with an estimated relatedness greater than 0.25, compared to those with an estimated relatedness value less than 0.25 for any of the estimators. A rarefaction analysis using RERAT described the ability of the 10 microsatellite markers used in the present study for accurately estimating relatedness (Figure S2). This analysis showed that change in relatedness had decreased to 0.038 using all 10 markers. A trend line, based on a power relationship (change in relatedness = $0.272*nloci^{-0.858}$; R2 = 0.999) indicated that increasing the number of loci to 20 would only decrease change in relatedness to 0.021, and increasing the number of loci to 100 would decrease change in relatedness to 0.005 (assuming loci had a similar level of polymorphism to the 10 loci used in this study).

Multiple dung piles

Of eight multiple dung piles, six contained only a single identical genotype. Of the two that were different, COLONY identified one of the dyads to be a first order relative (although couldn't distinguish between sibling or parent-offspring), and the other dyad to be a half-sibling.

Mating system

Mating system was investigated using the program COLONY to estimate relative numbers of half and full-sibships. Number of full siblings was one ($p = 0.999$) and number of half-siblings was 207 and 175 for posterior probability likelihoods of greater than 0.95 and greater than 0.80, respectively. This is highly indicative of a species that exhibits polygamy and or promiscuity.

Duplicate genotypes

All but one pairwise distance between identical genotypes was less than 1 km. The dyad that was greater than 1 km constituted two dung piles 25.5 km apart. Average distance between identical genotypes was 0.655 km (pairwise $n = 36$), or 0.103 km excluding the pair 25.5 km apart (pairwise $n = 35$). When classifying multiple dung piles as a single genotype, average distance between identical genotypes was 2.271 km (pairwise $n = 13$), or 0.337 km excluding the pair 25.5 km apart (pairwise $n = 12$).

Spatial genetic structure

STRUCTURE 2.3.4 [46] was unable to assign individuals to more than one population (data not shown). In addition, a mantel test was unable to detect any isolation by distance in the study area ($p = 0.462$, $r^2 = 0.000979$, scatterplot shown in Figure S1). These results show that the sampling area effectively constitutes a single random mating population without apparent subdivision.

Sex-biased dispersal

There were no significant F_{ST} values between North and South or East and West sides of the study area for either males or females. Mean relatedness in males was significantly lower than in females (males: -0.0478, females: -0.0065, $p < 0.01$, $t = -2.907$), indicating that males were less related than females presumably because of a higher male immigration rate into the study area. Haplotype diversity in males was higher than in females, true for both the 325 bp (males: 0.8772, females: 0.8250) and the 543 bp (males: 0.9286, females: 0.9048) fragments of mtDNA CR. As mentioned above, STRUCTURE 2.3.4 [46] was unable to assign individuals to more than one population. This was also true when only males or females were considered. mAIc for females was 0.85455, and for males was -0.91785 ($p < 0.05$). vAIc for females was 8.61963 and for males was 10.77515 ($p = 0.2809$).

DISCUSSION

This study aimed to elucidate information about okapi sociality, mating system and dispersal. Before this study was carried out, the only information available was some mixed reports on sociality [30,31,55]. Any information that can be added to the little that is currently known about this species is therefore of great benefit to the species conservation efforts.

Okapi sociality

There is a great deal of variation in social structure amongst ungulates, and even among ungulates sharing a similar distribution to okapi. Blue duikers (*Philantomba monticola*) form permanent pairs, occupying exclusive home-

ranges, whereas red duikers (*Cephalophus natalensis*) are solitary with greatly overlapping home-ranges [8]. Sitatunga (*Tragelaphus spekii*) are mostly solitary, however, do have a tendency to be gregarious for reasons related to food availability [10]. Bongo (*Tragelaphus eurycerus spp.*) form social groups of approximately 10-20 individuals, and groups have home ranges measured at between 19-49 km^2 [9]. Sociality was investigated in the present study for okapi using a combination of spatial autocorrelation analysis, relatedness estimators, and a description of the pattern of identical genotypes in the dataset. Spatial autocorrelation generally showed a pattern whereby there was negative autocorrelation at the larger distances (in the female, male and combined datasets), and positive autocorrelation at the shorter distances. Unexpectedly, there was a positive autocorrelation at 50 km for the male dataset. A possible explanation for this result could be high male sibling dispersal distances, although this hypothesis would need to be tested in future studies. There was also a negative autocorrelation at 0.6 km. This could be explained by proximity of unrelated male-female mating pairs. Unfortunately, this result (based on the male-female combined dataset) could not be tested directly with males and females separately (at 0 - 2 km), as these datasets were not large enough at this distance class. Our results therefore demonstrate a detectable correlation between geographic and genetic distance, at the scale of the RFO (maximum distance between samples 118.7 km), but only at specific distance categories. Also, the negative autocorrelations were usually at only the largest pairwise distances, implying a limited effect of isolation by distance operating just below the extent of the study area. The positive autocorrelation at ≤1 km for both males and females is evidence of social interaction between relatives at this small

spatial scale. As mentioned earlier, this dataset may contain juveniles, and so it is likely that these significant positive values are detecting small family groups with a low but detectable level of spatial association, similar to that described in Bodmer and Gubista (1988).

Dyads with a relatedness estimate of greater than 0.5 had an average geographic distance that was lower than that of the dyads with a relatedness estimator less than 0.5. This was true for all seven estimators implemented in COANCESTRY, although this difference was only significant in two cases. This finding suggests a relatively weak overall correlation between relatedness and geographic distance, but with significant associations at the highest relatedness values. Although the difference in geographic distance is significant, the magnitude of this difference is not particularly large (38.8 – 42.5 km vs 48.8 – 48.9 km). Taken together, the results of the spatial autocorrelation and relatedness patterns are indicative of a species where genetic structuring is determined more by relatively high dispersal ability, and a small proportion of spatially proximate dyads (for example mother offspring) than by a tendency to form tight social groupings.

Only one genotype was detected at six of the eight multiple dung piles from the study site. The other two were found to be relatives. This finding again appears to show that okapi form small family units, with no evidence for larger social groups of extended family members. The COLONY analysis was unable to distinguish between relationship classes for one of the dyads from the multiple dung piles, and the other dyad was a pair of half-siblings. The results from the multiple dung piles seems to indicate that large social stable units appear to be

very unlikely to be formed in this species. We can therefore accept our first hypothesis, that okapi are mostly solitary animals. This social strategy has been predicted as a means of animals avoiding predator detection [15,16], consistent with the ecology of okapi, which are known to be predated heavily by leopards [30].

Okapi mating systems

COLONY assigned one dyad to be full siblings (p = 0.999) and 207 and 175 half-siblings with posterior probabilities of greater than 0.95 and 0.80, respectively. We can therefore accept our second hypothesis, that okapi are *genetically* polygamyous or promiscuous. This is not unexpected, as monogamous mating systems occur in only ~5-15% of all mammalian species [20,56,57]. Also, even in predominantly monogamous animals, a detectable level of promiscuity often occurs [58-61]. Among the hypotheses advanced for the function of polygamy and promiscuity are that they may function as a means of reducing genetic incompatibility for a particular sex (usually females; [62]) or that they may be under selection on a particular sex (usually males) to dominate a large number of females [63]. Our results cannot rule out social monogamy in okapi, however do make this mating system much less likely. In addition, the rarity of social monogamy in mammals, and the findings of Hart and Hart [30] suggesting that male home-ranges overlap with several females, allow us to conclude that the mating system of okapi is most likely to be genetic and social polygyny or promiscuity.

It is worth mentioning that this mating system is highly dependent on the abundance and distribution of individuals, and relies on there being enough females occupying small enough adjacent territories to be defended by a single male [19,64]. This would be much more likely to be the case in the RFO, a region where okapi density is thought to be relatively high [27,65], although even in this habitat food appears to be a limiting factor [30]. Mating systems can vary within a species, depending on variations in resource distribution, predation pressure and costs of sociality [20,66,67]. These factors are likely to vary greatly across the okapis range, potentially leading to different mating strategies in different regions.

When classing multiple dung piles as a single genotype, average distance between identical genotypes was 2.271 km (pairwise n = 13), or 0.337 km excluding the largest movement event detected (25.5 km; pairwise n = 12). The duplicated genotypes, excluding the largest movement event, all fall well within even the smallest home-range size previously measured for okapi (Hart and Hart 1989). The movement event of 25.5 km was by a male, and represents the only *potential* dispersal event ever recorded for this species. This is a direct estimate of dispersal, and as such it cannot be determined if this corresponds to a successful dispersal event (i.e. resulted in a mating), or even if this move was a permanent one as it is possible that this individual moved to this location for a limited time and then returned. Nonetheless, this is valuable information as it clearly gives some indication of the movement potential of okapi.

The spatial autocorrelation analysis in the present study detected genetic structure, whereas IBD analysis did not. It is likely that the spatial scale investigated in this study is not large enough to detect a correlation between genetic distance and geographic distance, which would likely emerge if a larger spatial scale were investigated. The significant spatial autocorrelation results indicate a relationship between geographic and genetic distance that is only acting at certain distance classes. This signal may be lost in the IBD analysis, which simultaneously investigates all distance classes. Other studies have identified local genetic structure that is likely to have caused isolation by distance at large spatial scales (e.g. badgers; [68]).

Sex-biased dispersal

Male-biased dispersal is the norm for mammals [23], however, exceptions have been found. A notable example is the study of Zhan et al. [26] who concluded that giant pandas demonstrate female-biased dispersal, based on vAIc values, mean spatial distances between individuals, and estimates of relatedness, F_{ST} and population genetic structure. We can accept a hypothesis of male-biased dispersal in okapi, based on i) significantly lower pairwise relatedness in males than females within our study site, ii) higher haplotype diversity in males than females, and higher mAIc for females than males. Differences in F_{ST}, microsatellite based genetic structure and vAIc were not significant. The lack of significant difference between F_{ST} values may be due to the limited power of the statistic. It is not unusual for only a subset of these tests to give significant values (e.g. [26]), as they have variable power depending on demographic parameters specific to the sampled population, for example dispersal rate [50]. The

hypothesis of male-biased dispersal can still be accepted with confidence due to multiple lines of evidence pointing towards this fact. This information is vital for okapi conservation plans. Dispersal is one of the main drivers in species persistence, especially in spatially structured populations [69]. This will become an increasingly important factor to consider in okapi conservation plans if deforestation continues at the current rate in the DRC. Notably, the spatial autocorrelation also shows that there is a spatial association between both males and females at small distances (< 5km), showing that in okapi, both sexes exhibit some degree of social behaviour at small spatial scales. This pattern of positive spatial association for *both* males and females at small distance classes is a relatively common phenomenon (e.g. birds [70], badgers [68] and wombats [71]), but does not appear to obviate these species from demonstrating considerable sex-biased dispersal.

The present study has made an important first step in describing sociality, mating systems and dispersal for okapi. These ecological features have important evolutionary consequences [3,72,73], and is a requirement for effective conservation management [74]. This information is therefore crucial for the conservation of this elusive, endangered giraffid.

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