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Contrasting Historical and Recent Gene Flow among African Buffalo Herds in the Caprivi Strip of Namibia

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Abstract

Population genetic structure is often used to infer population connectivity, but genetic structure may largely reflect historical rather than recent processes. We contrasted genetic structure with recent gene-flow estimates among 6 herds of African buffalo (*Syncerus caffer*) in the Caprivi Strip, Namibia, using 134 individuals genotyped at 10 microsatellite loci. We tested whether historical and recent gene flows were influenced by distance, potential barriers (rivers), or landscape resistance (distance from water). We also tested at what scales individuals were more related than expected by chance. Genetic structure across the Caprivi Strip was weak, indicating that historically, gene flow was strong and was not affected by distance, barriers, or landscape resistance. Our analysis of simulated data suggested that genetic structure would be unlikely to reflect human disturbances in the last 10–20 generations (75–150 years) because of slow predicted rates of genetic drift, but recent gene-flow estimates would be affected. Recent gene-flow estimates were not consistently affected by rivers or distance to water but showed that isolation by distance appears to be developing. Average relatedness estimates among individuals exceeded random expectations only within herds. We conclude that historically, African buffalo moved freely throughout the Caprivi Strip, whereas recent gene flow has been more restricted. Our findings support efforts to maintain the connectivity of buffalo herds across this region and demonstrate the utility of contrasting genetic inferences from different time scales.

Key words: *African buffalo, connectivity, dispersal, gene flow, microsatellite, Namibia*

Conservation efforts require both understanding current processes and appreciating past processes, which may have shaped current species distributions. Connectivity among populations, in particular, is believed to have changed dramatically in many systems due to habitat fragmentation or human-built barriers. Connectivity is increasingly considered a key component of conservation efforts on large landscapes, especially for mobile and wide-ranging animal species (Hilty et al. 2006), and is critical for maintaining genetic diversity and ensuring persistence of small populations, maintaining species throughout their historic range, and facilitating dispersal and seasonal migrations. Efforts to characterize animal movements have included short- or long-term monitoring of adults or dispersing juveniles (e.g., Peacock and Smith 1997), estimating dispersal rates and distances (Nathan et al. 2003), and estimating gene flow among populations (e.g., Epps et al. 2005). Each method allows inferences at particular temporal and spatial scales (Lowe and Allendorf 2010); thus, evaluating connectivity across time scales could inform our understanding of changes in landscape connectivity and improve connectivity conservation.

In this study, we address connectivity at multiple time scales by contrasting genetic structure and recent gene flow among herds of African buffalo (*Syncerus caffer*) distributed across the Caprivi Strip of Namibia (Figure 1), which is of central importance to the proposed Kavango-Zambezi (KAZA) Transfrontier Conservation Area. African buffalo exemplify many of the challenges of conservation on large landscapes such as the KAZA, which is a patchwork of different land uses, tenure types, ethnic groups, cultures, and boundaries,

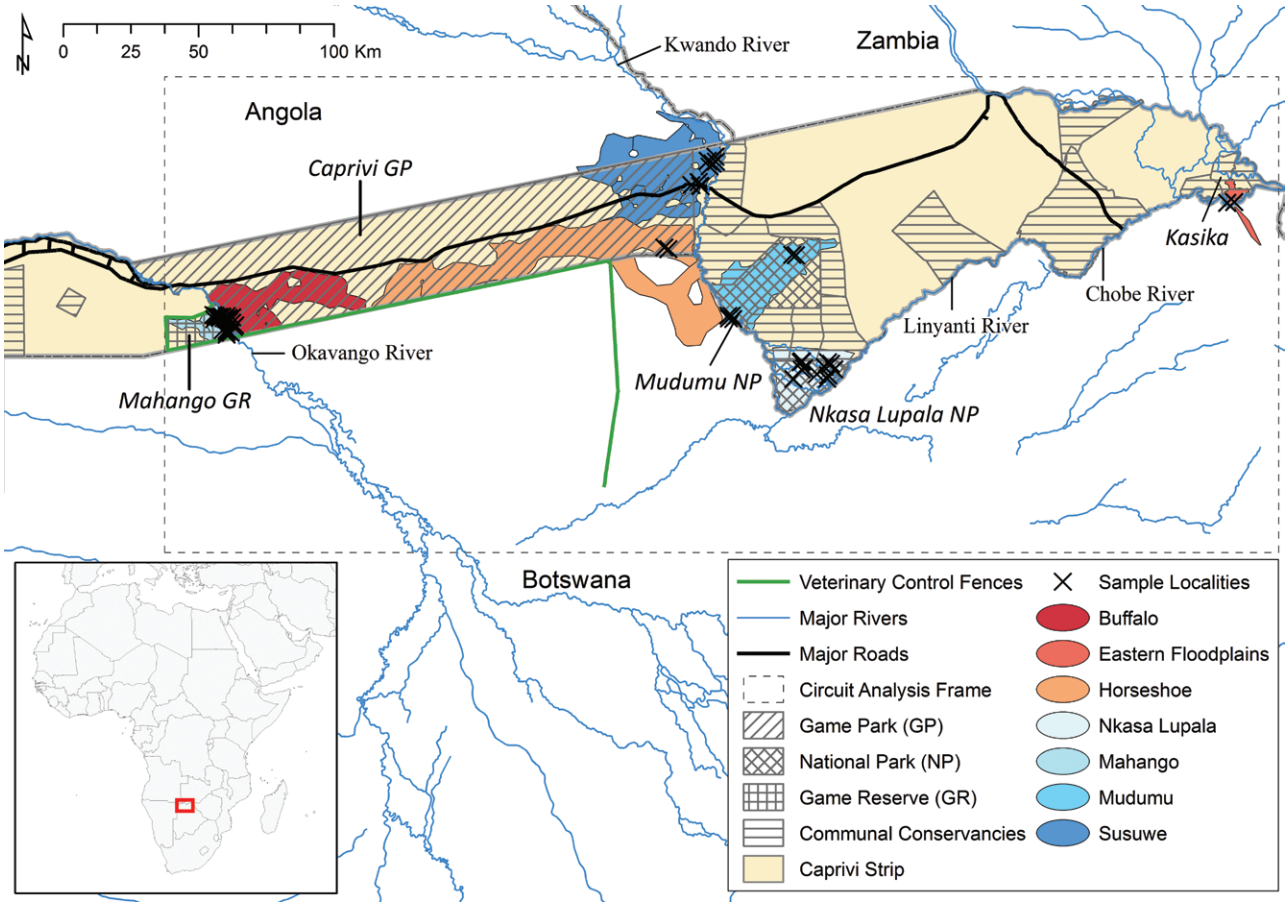

Figure 1. Locations of population genetic samples from African buffalo in the Caprivi Strip, Namibia, with Local Convex Hull 100% home ranges (colored polygons) generated from 5 to 8 GPS-collared individuals from each study site. Samples from Horseshoe and Susuwe were combined into a single herd for genetic analyses because of spatial overlap between home-range polygons in those 2 areas. Major rivers, veterinary control fences, and protected areas are also depicted, as well as the analytical frame for circuit analysis.

making transboundary management of natural resources a key issue. Given their economic (Naidoo et al. 2011) and ecological (Sinclair 1977) importance, buffalo have also been identified as a logical resource around which to start building cross-border institutions for natural resource management among KAZA nations (Martin 2003). However, there are numerous apparent constraints to buffalo movement across the Caprivi Strip. Rivers and arid regions far from rivers may have restricted gene flow historically, whereas in the 20th century, agricultural control fences and expanding human settlement may have limited buffalo movements (Naidoo et al. 2012a). Efforts to restore connectivity in this and other landscapes would benefit from better understanding both historical (to help understand appropriate conservation targets) and present-day movement patterns.

African buffalo move widely in response to changing environmental conditions (Ryan et al. 2006), are important prey for large carnivores and a prized game species (Sinclair 1977), and are involved in disease transmission to and from domestic cattle (Thomson 2009). Thus, movement potential of African buffalo has been studied at scales ranging from home ranges and investigations of habitat use (Winnie et al. 2008; Cornelis et al. 2011) to evaluations of genetic structure conducted at regional to continental scales (Simonsen et al. 1998; Van Hooft et al. 2000; Van Hooft et al. 2002; Heller et al. 2010). African buffalo populations demonstrate the weakest genetic structure of any African ungulate yet studied, even at continental scales (Lorenzen et al. 2008). Simonsen et al. (1998) argued that connectivity among African buffalo populations should be conserved because such weak structure likely had been maintained by high gene flow among regions. However, to our knowledge, only 1 study to date (Van Hooft et al. 2003) has evaluated genetic structure among multiple African buffalo populations (sometimes defined as herds, as in this study) at relatively local scales, where most attempts to conserve connectivity occur. That study detected significant genetic structure in mitochondrial DNA among herds separated by as little as 6 km in East Africa, whereas in Kruger National Park (South Africa), 5 herds separated by 8–240 km were not genetically differentiated

at mitochondrial or nuclear markers (14 microsatellite loci, Van Hooft et al. 2003). Therefore, the degree of local genetic structure and thus the size and historical levels of gene flow among populations can vary widely depending on location, and prior connectivity of African buffalo populations should not be assumed even at local scales.

Here, we evaluate 1) the degree of genetic structure among buffalo herds that we defined on the basis of movement data and land management and whether genetic structure is predicted by distance, potential riverine barriers, or landscape-resistance hypotheses based on distance to water; 2) recent gene flow among buffalo herds and whether recent gene-flow estimates show evidence of a changing relationship between gene flow and distance, barriers, or landscape resistance; 3) the scale of spatial autocorrelation of relatedness among individuals; and 4) how contrasts between genetic structure (reflecting long-term patterns of gene flow) and recent gene flow inform our understanding of past connectivity of buffalo populations. Finally, we discuss the relevance of our findings with respect to current efforts to understand and maintain connectivity of buffalo herds across the Caprivi Strip.

Materials and Methods

Genetic Data

We extracted DNA from 138 blood samples of African buffalo captured in 2007 and 2009 in all of the major buffalo herds in the Caprivi Strip. DNA was extracted using Qiagen Tissue DNA extraction kits (Qiagen Inc., Valencia, USA) or phenol–chloroform methods. We optimized polymerase chain reaction (PCR) conditions for 12 fluorescently labeled primer pairs for bovine and ovine microsatellite loci previously employed in published analyses of African buffalo (Heller et al. 2010; Van Hooft et al. 1999) (Supplementary Appendix S1). We chose those primer pairs on the basis of high variability, low amplicon size (in case DNA was degraded), and position on different bovine/ovine chromosomes to decrease the likelihood of linkage disequilibrium. We used 0.6 μL of template DNA in 15 μL PCR, with 10 μg bovine serum albumin, 2.25 mM $MgCl_2$, 0.16 mM each dNTP, 1× Apex PCR buffer, 0.1 μM (1.5 pmol) each primer, and 0.7 units Apex® Hot Start Taq polymerase (Genesee Scientific, San Diego, USA). We used a 15-min 95°C initial activation step, followed by 39 cycles denaturing at 95°C for 30 s, annealing at temperatures specific to each locus (Appendix 1) for 45 s, and extending at 72°C for 30 s. We used an ABI 3730 DNA sequencer and GeneMapper to identify and estimate the size of alleles (Applied Biosystems, Carlsbad, CA, USA). We repeated amplification of samples up to 3 times at any locus that failed to amplify or had alleles that could not be clearly identified. We excluded any samples that failed to amplify or gave inconsistent results at more than 2 loci after 3 repeat PCRs.

Many studies evaluating the effects of landscape on gene flow have used individual-based analyses to avoid sometimes artificial definitions of "populations" (e.g., Cushman et al. 2006). However, because African buffalo congregate in large breeding herds that range over large areas, especially in the wet season, and because we sampled many individuals from the same herds at the same locations, we decided that population (herd)-level analyses of gene flow were most appropriate. We used satellite telemetry data (presented fully in Naidoo et al. 2012a; Naidoo et al. 2012b) to inform our definition of herds as follows. We grouped samples initially into 7 populations ("herds") relating to initial capture sites that were defined on the basis of putative barriers such as roads and rivers, inhospitable intervening habitat (such as cultivated areas), protected area status, and geographic distance (Table 1; Figure 1). All herds had multiple capture sites, and multiple individuals were captured at each site. We used GPS locations from 31 individuals (total across all sites) during 2007–2011 to calculate herd-level home ranges for each of these 7 sites, grouping all individuals within a capture site and using the Local Convex Hull (LoCoH) home-range estimator to produce 100% isopleth home ranges (Getz et al. 2007). The resulting home ranges were consistent with the view that herds were geographically separated from one another at the time scales at which our tracking collar data were collected and showed no overlap among individuals from different sites, with the exception of Susuwe and Horseshoe (Figure 1). Because individuals from those sites overlapped in range and had a wide area of contact, we grouped them into a single herd (hereafter referred to as "Susuwe/Horseshoe") for further analyses.

After defining the 6 study herds, we used Arlequin version 3.11 to test for linkage disequilibrium and deviation from Hardy–Weinberg equilibrium (HWE) at each locus in each herd, as well as to calculate expected heterozygosity and average numbers of alleles per locus. We used Genepop to assess estimates of F_{IS}, F_{IT}, and F_{ST} by Weir and Cockerham (1984) and to test for deviation from HWE across loci in each herd.

Estimating Genetic Structure (Historical Gene Flow) Among Herds

We calculated population pairwise F_{ST} values (Φ_{ST} by Weir and Cockerham 1984, hereafter referred to as F_{ST}) among all

Table 1 Average expected heterozygosity (H_e) and allelic richness of African buffalo herds in 6 sampling areas across 10 microsatellite loci

Sampling herd	Sample size	Average number of alleles/locus	H_e	Average allelic richness[a]
Buffalo	16	7.7	0.78	7.3
Eastern floodplains	13	7.7	0.79	7.7
Mahango	21	8.1	0.77	7.3
Nkasa Lupala	36	9.5	0.81	7.6
Mudumu	16	7.3	0.78	7.0
Susuwe/ Horseshoe	32	8.7	0.80	7.3

[a]Subsampled based on the smallest sample size (n = 13) to correct for differences in sample size.

6 herds as a measure of genetic structure (genetic distance) using Genepop and assessed whether those values differed from 0 (no differentiation) using Fisher's exact test for differentiation of alleles (Ryman et al. 2006). We estimated 95% confidence intervals for F_{ST} using FSTAT. We used Software for the Measurement of Genetic Diversity (SMOGD) (Crawford 2010) to estimate G'_{ST} among population pairs as an alternate metric of genetic structure (and historical gene flow) that is less influenced by marker variability (Hedrick 2005). Because population pairwise F_{ST} values typically change slowly for large populations (Wang 2004), and the total buffalo population in the Caprivi Strip was estimated at greater than 3000 during the 1990s (Martin 2003), we interpreted F_{ST} to be primarily an index of historical gene flow among populations, with relatively little influence from migration rates within the last few generations (Balkenhol et al. 2009). To evaluate this interpretation, we used EasyPop (Balloux 2001) to investigate the number of generations required to detect changes in population pairwise F_{ST} among the 5 native study populations after a 10-fold decline in migration rate and a 2-fold decline in dispersal distance (Supplementary Appendix S1). We also estimated how many generations would be required for the system to reach a new equilibrium level of genetic structure (Supplementary Appendix S1).

Estimating Recent Gene-Flow Among Herds

We used BIMr (Faubet and Gaggiotti 2008) to estimate rates of recent gene flow between herd pairs (i.e., the proportion of alleles that were derived in the previous generation from the other herd). BIMr uses assignment tests to estimate migration rates within the last generation by assuming drift-migration equilibrium at the previous generation. BIMr performs best when migration rates are high but global F_{ST} values exceed 0.01; thus, this technique may give greater ability to discern variation in gene flow when genetic structure is weak (Faubet and Gaggiotti 2008). Because of overlapping generations and the high likelihood that natural populations are not in drift-migration equilibrium at any recent time step, we interpreted BIMr estimates as a relative index of recent gene flow rather than a precise estimate of gene flow in the previous generation. We used a burn-in period of 20 000 followed by 1 000 000 iterations for each run, then averaged population pairwise estimates across 10 runs. We estimated 95% confidence intervals using the averaged estimates for 10 runs. We repeated this exercise on the simulated data sets used to evaluate changes in genetic structure (described above) to determine whether BIMr could discriminate recent changes in gene flow for the sample sizes, numbers of populations, and number of microsatellite loci employed in this study.

Testing Explanatory Models for Genetic Structure and Gene Flow

We tested whether genetic and geographic distances between herds were correlated using 2 alternative measures of distance. First, we estimated distance between the edges of polygons drawn around the locations where genetic samples were collected within each herd. Second, we estimated distance between the edges of home-range polygons estimated from 5 to 8 GPS-collared individuals in each sampled herd (Figure 1). In both cases, rather than simply using straight-line distances, we used Circuitscape (Shah and McRae 2008) to estimate cumulative resistance (McRae et al. 2008) among herd pairs, using a resistance layer where all values were set to 1. This approach estimates cumulative resistance over all possible paths linking 2 locations and thus can distinguish between 2 locations connected by narrow versus wide paths. We used that measure of distance to allow direct comparison with landscape-resistance models tested in Circuitscape (see below). We defined our analytical landscape for Circuitscape using a rectangle large enough to encompass the entire Caprivi Strip and the river systems to the North and South that could facilitate East–West gene-flow movement but limited the southwestern extent by including the livestock fence as a complete barrier to movement (Figure 1). We used XLSTAT to conduct Mantel tests (10 000 permutations, Smouse et al. 1986) to test whether genetic distance was positively correlated with either measure of geographic distance (i.e., isolation by distance) and used the best-supported measure for further comparisons.

We also tested whether genetic structure was correlated with cumulative resistance estimates (i.e., isolation by resistance, McRae 2006) for 2 sets of models based on hypotheses of landscape barriers or resistance (Supplementary Table S2). Those hypotheses included 1) major rivers (e.g., Okavango and Kwando/Linyanti/Chobe Rivers) were a barrier to historical and recent gene flow, based on our observation that only 1 of 31 collared buffalo crossed a river in 4 years of monitoring (Figure 1); and 2) historical and recent gene flow were facilitated by proximity to rivers, as buffalo are constrained to areas near open water, especially in the dry season (Ryan et al. 2006; Cornelis et al. 2011; Naidoo et al. 2012a). For each hypothesis, we generated a series of resistance models with increasing resistance values for hypothesized barriers, low-quality habitat, or distance from rivers (Supplementary Table S2). That set of models allowed us to test variants of each basic hypothesis rather than assuming a single a priori relationship with gene flow (Shirk et al. 2010). For each resistance model, we used Circuitscape (Shah and McRae 2008) to estimate cumulative resistance between herd pairs as done for the distance-only model (above), creating a matrix of pairwise cumulative resistances between each herd pair for each model.

Next, we evaluated correlation of each resistance matrix from those models with the matrix of genetic distances between herd pairs. We used a systematic model testing approach (e.g., Cushman et al. 2006; Shirk et al. 2010) in which 1) we used simple Mantel tests to determine (within a single hypothesis set) which model was most strongly correlated with gene flow; 2) we used the model from each hypothesis set with the strongest correlation with gene flow to compare with the correlation of the isolation by distance model; and 3) if any model in step 2 was significantly correlated with gene flow, we used partial Mantel tests to test whether the best model from each set explained variation in genetic distance

after controlling for correlation with distance (Cushman and Landguth 2010). We predicted that any valid landscape-resistance model would exhibit correlation with genetic distance even after controlling for geographic distance and would result in lower correlation between genetic distance and geographic distance while controlling for resistance. This approach greatly increases the chance of identifying the correct explanatory model (Cushman and Landguth 2010).

We also used posterior probabilities for explanatory models (estimated in BIMr) to evaluate the effect of distance in comparison with a null model (no geographic explanation) on recent gene-flow rates. By that method, which performed well with simulated data (Balkenhol et al. 2009), explanatory models are used as priors for estimating recent gene flow and then evaluated post hoc. Landscape barrier or resistance models were not tested by this method unless identified as potentially explanatory in the previous analyses.

Finally, we repeated the Mantel/partial Mantel analytical approach using recent gene-flow estimates instead of genetic distance (reflecting historical gene flow), with 2 modifications. First, when calculating cumulative resistance values between herds for distance, barrier, and landscape-resistance models, we included the livestock control fences on the southern side of the Caprivi Strip by assigning a very high resistance value of 100 000 to the location of the fence. That fence was built c. 1960 and would have constrained all subsequent movements by buffalo to the southwest. Second, because plotting estimates of recent gene flow against distance revealed an apparent outlier, likely resulting from a specific barrier to gene flow between 2 herds (see results; Figure 2b), we removed that comparison and retested for isolation by distance. We used that restricted data set for the subsequent testing of landscape resistance and barrier hypotheses.

Testing for Spatial Autocorrelation in Relatedness Among Individuals

Although the clumped nature of buffalo herds and the genetic samples made population-level analyses most appropriate in this system, we also evaluated spatial autocorrelation of pairwise relatedness coefficients among individuals to determine the smallest spatial scales at which genetic structuring was detectable. We used Spagedi (Hardy and Vekemans 2002) to estimate coefficient of relatedness (r) by Lynch and Ritland (1999) among all 118 individuals in protected areas excluding Mudumu. Relatedness typically varies from 0 (unrelated) to 1 (identical), although negative estimates are possible and may be interpreted as individuals even less similar than expected by chance. We used Spagedi to test for spatial autocorrelation among individuals at increasing distance classes; distance classes were of variable width to maintain relatively equivalent numbers of comparisons, and the largest class included the maximum extent of the study area (Supplementary Figure S3). We estimated average relatedness ("jackknifed" across loci by removing each locus and recalculating, and then averaging estimates) for comparisons within each distance class and tested whether those values exceeded (if positive) or were less than (if negative) null expectations using 10 000 permutations. We used 10 distance classes but repeated the analysis using 15 and 20 classes to ensure that results were not strongly affected by the classification scheme.

Results

Genetic Data

Four samples were excluded from further analysis because they were degraded by postcapture handling and failed to amplify at more than 2 loci. Of the remaining 134 samples in the final data set, 12 samples were missing data at 1 locus and 1 was missing data at 2 loci.

Of the 12 loci we genotyped, 1 locus (INRA123) showed significant deviations from HWE in 5 out of 6 herds. That locus had an F_{IS} value of 0.56, far higher than values for all other loci (data not shown), suggesting null alleles were present. We excluded that locus from further analyses to avoid biased estimates of genetic structure. One other locus (TGLA057) showed deviation from HWE (in 1 herd only) after applying a Bonferroni correction for multiple tests but was not excluded. No consistent patterns of linkage disequilibrium between loci were observed across herds. However, we excluded 1 additional locus (BM4028) from our analyses of genetic structure and gene flow because that locus had very strong stutter bands and an unusually high proportion of apparent microvariant alleles that were difficult to size consistently. Before excluding those 2 loci, HWE tests across loci by population were significant for 3 of 6 populations; after excluding those 2 loci, only Nkasa Lupala showed significant deviation from HWE (i = 0.0014). Total alleles per locus across the entire data set varied from 5 to 20; expected heterozygosity (H_e) per locus varied from 0.49 to 0.87 (Supplementary Table S1). Within herds, over the 10 loci used in analyses, average H_e varied from 0.77 (Mahango) to 0.81 (Nkasa Lupala). Average allelic richness (corrected for sample size) varied from 7.0 to 7.7 alleles/locus (Table 1). For the overall data set of 134 individuals at 10 loci, we estimated F_{IT} at 0.026, F_{ST} at 0.007, and F_{IS} at 0.019.

Genetic Structure and Recent Gene Flow Among Herds

Genetic structure among African buffalo herds across the Caprivi Strip was weak, with population pairwise F_{ST} values varying from 0 to 0.019 (Table 2) and G'_{ST} values varying from 0 to 0.078 (Supplementary Table S3). After correcting for multiple comparisons, we detected significant differentiation (i.e., $F_{ST} > 0$) between 4 pairs of herds (Table 2). In some cases, herds with 100% home-range polygons separated by only a few kilometers showed detectable genetic structure (e.g., Susuwe/Horseshoe-Buffalo, Table 2, Figure 1). Buffalo from the easternmost portion of the Caprivi Strip, on the Eastern floodplains, were not significantly differentiated from any other herds despite being the most distant herd. Low sample size in that area (n = 13, Table 1) resulted in low power for those tests, although the estimates themselves are not expected to be biased. As expected, buffalo in Mudumu

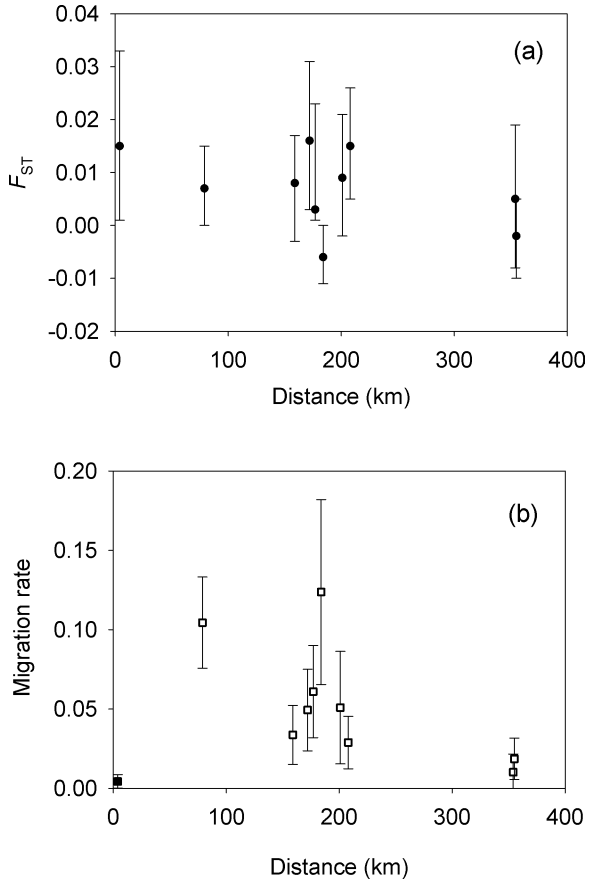

Figure 2. Pairwise estimates of F_{ST} (**a**; an index of long-term gene flow, estimated using GenePop, with 95% confidence intervals) and recent gene-flow estimates (**b**; estimated using BIMr, with 95% confidence intervals) plotted against distance (km) among African buffalo herds in the Caprivi Strip, Namibia. Genetic distances (**a**) were not positively correlated with geographic distance as predicted under the isolation by distance model (Mantel test, $r = -0.63$, $P = 0.062$). However, recent gene flow (**b**) declined with distance as predicted under the isolation by distance model (Mantel test, $r = -0.75$, $P = 0.022$) when the Mahango to Buffalo comparison (filled black square) was excluded.

National Park, introduced in the 1980s using buffalo from Nkasa Lupala National Park, were not significantly differentiated from buffalo in Nkasa Lupala (Table 2). There was no significant reduction in allelic richness resulting from the translocation (paired *t* test, $t = -1.33$, $P = 0.10$). Estimates of recent gene flow using BIMr (in theory, the proportion of alleles derived from migrants in the previous generation but best interpreted as a relative index) varied from relatively low (Buffalo-Mahango, <1%, Table 2) to quite high (Nkasa Lupala-Susuwe/Horseshoe, ~10%, Table 2).

Simulations (Supplementary Appendix S1) using sample sizes, locus numbers, and population parameters emulating those observed in the real data demonstrated that even for relatively small herds (census size ~400), reducing migration rates 10-fold and halving dispersal distances caused relatively slow changes in genetic structure (F_{ST}). Mean F_{ST} increased over time from 0.006 (no change in migration or dispersal) to 0.031 after 5 generations, 0.047 after 10 generations, and 0.071 after 20 generations of reduced migration and dispersal (37.5, 75, and 150 years assuming 7.5 years/generation; Supplementary Figure S1a). At least 162 generations (1335 years) were required for F_{ST} to reach equilibrium (here, determined using the mean value observed in the last 500 generations of the simulation, when estimates appeared to be relatively stable), although F_{ST} reached near-equilibrium levels within 50 generations (375 years; Supplementary Figure S2). Larger populations (as likely occurred prior to the rinderpest epidemics of the late 19th century) would change more slowly. Migration rates estimated among simulated populations using BIMr showed sharp and detectable decreases after only 1 generation (Supplementary Figure S1b).

Testing for Isolation by Distance and Isolation by Resistance

We did not detect isolation by distance (either among sampling areas or home-range polygons) in our index of historical gene flow (F_{ST}; Table 3, Figure 2a). None of the hypotheses of isolation by barrier or resistance (Supplementary Table S2) was supported, indicating that those factors did not have a consistent effect on long-term gene flow across the study area (Table 3 and Supplementary Table S4). Resistance from the rivers as barrier model was negatively correlated with genetic structure, opposite to our prediction, but that effect was not supported by partial Mantel tests (Supplementary Table S4), suggesting that it was a spurious correlation.

Estimates of recent gene flow (BIMr; Table 2) among herds were likewise not correlated with distance metrics (Table 2) or barrier or resistance models (Supplementary Table S4), and the null model was favored over geographic distance in 10 of 10 analytical runs in BIMr (average posterior probabilities were 0.73 and 0.27 for null and geographic distance models, respectively). However, gene flow decreased with increasing distance for all herd comparisons except one (Figure 2b): gene flow was extremely low between the Buffalo and Mahango herds, only 4 km apart, but separated by the Okavango River. After excluding that point, under the assumption that the Okavango River acted as a unique barrier to gene flow, we detected isolation by distance (IBD; Table 2). Without removing that point, the strictest rivers as barrier model approached significance (Supplementary Table S4), but the effect was largely driven by that single outlier.

After repeating tests for isolation by barriers and resistance in recent gene flow with the Buffalo-Mahango comparison excluded, simple Mantel tests did not identify any resistance or barrier model that performed better than isolation by distance (Supplementary Table S4). Partial Mantel tests (Supplementary Table S4) revealed that the resistance and barrier models, although correlated with recent gene flow, had no support after controlling for correlation with distance.

Table 2 Pairwise estimates of genetic distance (F_{ST}; below diagonal) and recent gene flow (proportion of alleles derived from migrants in the previous generation; above diagonal) among African buffalo herds in the Caprivi Strip, Namibia, derived from 10 microsatellite loci

	Buffalo	Eastern flood plains	Mahango	Nkasa Lupala	Mudumu	Susuwe/ Horseshoe
Buffalo		0.010	0.004	0.051	NA[a]	0.049
Eastern floodplains	0.005 (0.610)		0.019	0.034	NA	0.124
Mahango	**0.016 (0.009)**	−0.002 (0.795)		0.029	NA	0.061
Nkasa Lupala	**0.009 (0.005)**	0.008 (0.070)	**0.015 (0.0002)**[b]		NA	0.104
Mudumu	0.003 (0.116)	0.0005 (0.596)	0.010 (0.332)	0.002 (0.271)		NA
Susuwe/Horseshoe	**0.016 (0.0007)**[b]	−0.006 (0.770)	**0.011 (0.0009)**[b]	**0.007 (0.0004)**[b]	0.00008 (0.313)	

Population pairwise F_{ST} values, with P values for tests of genic differentiation (in parentheses, bold if $P < 0.05$), were estimated using GenePop (Rousset 2008); recent gene flow was estimated using BIMr (Faubet and Gaggiotti 2008). G'_{ST} values are reported in Supplementary Table S3.

[a]We did not estimate recent gene-flow rates for Mudumu because that herd was established by recent translocation and thus could have confounded prior probability estimates in BIMr based on geographic distance.

[b]Significant after sequential Bonferroni correction for multiple comparisons ($P < 0.0033$).

Table 3 Mantel tests of correlation of pairwise estimates of genetic distance and recent gene flow with distance between herd centers and home-range edges among African buffalo herds in the Caprivi Strip, Namibia

Data set	Gene-flow estimates and explanatory models tested for correlation	Correlation coefficient (r)	Significance (P)
Full	genetic distance (F_{ST}) × distance (between sampling polygons)	−0.56[a]	0.094
Full	genetic distance (F_{ST}) × distance (between edges of home-range polygons)	−0.64[a]	0.054
Full	Recent gene flow (BIMr) × distance (between sampling polygons)	−0.35[b]	0.313
Full	Recent gene flow (BIMr) × distance (between edges of home-range polygons)	−0.37[b]	0.283
Mahango-Buffalo excluded	**Recent gene flow (BIMr) × distance (between sampling polygons)**	**−0.76**[b]	**0.020**
Mahango-Buffalo excluded	**Recent gene flow (BIMr) × distance (between edges of home-range polygons)**	**−0.66**[b]	**0.037**

Correlations with $P < 0.05$ are bolded. Tests of genetic structure using G'_{ST} (data not shown) were likewise not significant.

[a]This correlation has the opposite trend than predicted by isolation by distance.

[b]This correlation has the expected trend as predicted by isolation by distance or resistance.

Genetic Distance and Relatedness Among Individuals

We detected positive spatial autocorrelation in pairwise estimates of relatedness (r) among individuals in the 3–9.5 km distance class (based on 10 distance classes; Supplementary Figure S3). Average r in all other distance classes did not differ from the null expectation or was lower (negative spatial autocorrelation, 152–172 km). Thus, animals sampled at locations 3–10 km apart were more closely related than more distant samples. Analyses using 15 and 20 distance classes were similar (15 classes, $r > 0$ at 4.4–9.4 km; 20 classes, $r > 0$ at 3–5.5 and 5.5–9.5 km).

Discussion

African buffalo herds across the Caprivi Strip showed generally low levels of genetic differentiation, although genetic structure was detectable among 4 of 15 pairwise tests (Table 2 and Supplementary Table S3). Unlike other studies (see below), we detected genetic structure (albeit at a low level) based on nuclear DNA markers between a pair of herds only a few kilometers distant, in this case situated on opposite sides of a major river (Buffalo-Mahango, Table 2). Ability to distinguish genetic structure (i.e., genetic distance estimates significantly greater than 0) is strongly influenced by sample size and number of loci evaluated, and statistically significant differences do not necessarily imply that biologically important differences exist. However, sample sizes in our study were comparable with or lower than those of other studies of African buffalo, (e.g., Van Hooft et al. 2003; Heller et al. 2010), so it is unlikely that we had additional power to detect structure. Most importantly, given the low levels of structure, it is clear that gene flow historically was high among all these populations.

As observed in other studies of genetic structure across eastern and southern Africa at scales of less than 500 km (Grobler and VanderBank 1996; Simonsen et al. 1998; Van Hooft et al. 2000; Van Hooft et al. 2003; Heller et al. 2010),

there was no relationship between genetic structure (F_{ST} or G'_{ST}) and geographic distance (see Figure 2 in Van Hooft et al. 2000). Because of the predicted lag in detectable genetic structure after population perturbations (lasting many generations for large populations, Balkenhol et al. 2009), the weak genetic structure and lack of isolation by distance we observed is best interpreted as evidence for strong connectivity of buffalo herds across the region within historical (but not necessarily present day) time frames. Our simulation experiments showed high variability but demonstrated that for populations of the size currently observed in Caprivi, at least 10–20 generations (75–150 years) would be required for strong decreases in gene flow to be reflected in overall F_{ST}; at least 50 generations (375 years) would be required to reach values near-equilibrium levels of genetic structure (Supplementary Figure S2). Historically, some populations may have been much larger than what we simulated (e.g., northern Botswana), meaning that genetic structure would take even longer to reach equilibrium (Wang 2004).

Recent gene flow among herds has been more restricted than historical gene flow (Figure 2). Recent gene flow was generally low among herds separated by greater than 200 km (Figure 2b) and thus appears to have occurred in more step-wise fashion than did historical gene flow. The known constraints to buffalo movement in this region, such as control fencing (Figure 1), may partly explain that pattern. Alternatively, this difference may have resulted in part from the different levels of resolution at inferring gene flow for these methods: when genetic structure is weak, assignment-based methods (e.g., BayesAss, BIMr) may be more reliable (Faubet and Gaggiotti 2008). However, our spatially explicit simulations of buffalo populations (Supplementary Appendix S1 and Figure S1) confirmed that recent gene-flow estimates changed much more rapidly than genetic structure after a decrease in migration rates and dispersal distance.

We detected even finer-scale relationships using individual relatedness estimates, which showed positive spatial autocorrelation among individuals sampled at locations separated by 3–10 km (Supplementary Figure S3). High pairwise estimates of relatedness (suggesting close relationships among individuals) occurred mostly within herds and therefore probably reflected herd associations. Dispersal between herds, even in the absence of barriers, is likely more difficult to detect by this method as a large number of related pairs would be required to create spatial autocorrelation. However, this analysis supports the evidence from satellite telemetry that the herds defined here behave as relatively discrete units, even though home-range polygons of individuals within different herds are in close proximity at times (Figure 1).

As expected when gene flow among populations is high, genetic diversity (expected heterozygosity, H_e) was similar among herds (Table 1). There was no evidence that a significant reduction in genetic diversity occurred in Mudumu after that herd was reestablished using animals translocated from Nkasa Lupala (as observed in some translocations of other species, e.g., Mock et al. 2004). However, in all these herds, genetic diversity was as high as or exceeded most reported values for African buffalo populations in eastern, central, or southern Africa. Using similar sets of microsatellite loci, Heller et al. (2010) reported H_e values ranging from ~0.75 to 0.82 in Kenya and Uganda, and Van Hooft et al. (2000) reported H_e = 0.66–0.81 in populations from southern Africa, East Africa, and Gabon. We conclude that none of the Caprivi Strip herds has been greatly isolated for many generations and that large population sizes (at least until recently) and high gene flow have maintained high genetic diversity.

Both genetic data and satellite telemetry suggested that rivers can act as barriers to movement and gene flow but do not do so uniformly. We found that gene flow was low between 2 herds only a few kilometers apart but separated by the Okavango River (Buffalo-Mahango, Figures 1 and 3), but models treating rivers as barriers across the entire study area did not explain variation in historical and recent gene flow (Supplementary Table S4). Satellite telemetry data (Figure 1) suggested that the Okavango River and the Kwando and Linyanti rivers are not often crossed by buffalo, but 1 individual was observed crossing the Chobe River in the Eastern Floodplains herd. The ability of buffalo to cross such rivers is likely driven by seasonal patterns of water flow and the size and depth of each river, which vary both spatially (e.g., the Chobe river in some parts is very shallow, and buffalo are observed to cross fairly regularly at the extreme eastern end of the Caprivi Strip) and temporally (in very dry years water levels may be low enough that even river stretches that are too fast and deep to normally be crossed may see buffalo movements). However, the western-most herd (Mahango) shows higher estimates of recent gene flow with other more distant herds to the east. That finding may contradict the interpretation that the Okavango has been a barrier to gene flow in recent times but also could be explained by gene flow between buffalo in northern Botswana and the central portion of the Caprivi Strip, or the population history of the Buffalo herd. Historically, herds on either side of the Okavango River would have been connected to large populations of African buffalo in the Okavango Delta of northern Botswana, where movement across the Okavango would have been facilitated by the breakup of the river into smaller channels, allowing buffalo in that region to interact with herds farther east in the Caprivi Strip. Thus, gene flow across the entire region may have been strongly influenced by buffalo populations outside of the Caprivi Strip, which are now mostly isolated by the Botswana–Namibia border fence in the western portion of the study area.

The weak patterns of genetic structure we observed suggest that historically, buffalo interacted either directly through home-range movement or dispersal across the entire Caprivi Strip or indirectly through interactions across the study area with large populations of buffalo in northern Botswana (Figure 2a). For instance, the Eastern Floodplains herd was little differentiated from even distant herds to the west (Table 2). In contrast, recent gene flow appears to have been more limited and occurred in "step-wise" fashion via gene flow among adjacent herds (Figure 2b). This difference may reflect recent anthropogenic fragmentation such as human settlements, cultivated areas, and construction of veterinary fences that have impeded buffalo movement. Thus,

our findings make it clear that the restricted movements exhibited by buffalo herds in some of these sampling areas (Naidoo et al. 2012a,b) stand in stark contrast to the signature of relatively unrestricted gene flow in the past. Comparing recent and longer-term estimates of gene flow may be a useful way to prioritize connectivity conservation efforts in this and other systems where historical patterns of movement are poorly understood.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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References

Balkenhol N, Gugerli F, Cushman SA, Waits LP, Coulon A, Arntzen JW, Holderegger R, Wagner HH. 2009. Identifying future research needs in landscape genetics: where to from here? Landscape Ecol. 24:455–463.

Balkenhol N, Waits LP, Dezzani RJ. 2009. Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. Ecography. 32:818–830.

Balloux F. 2001. EASYPOP (version 1.7): a computer program for population genetics simulations. J Hered. 92:301–302.

Cornelis D, Benhamou S, Janeau G, Morellet N, Ouedraogo M, de Visscher MN. 2011. Spatiotemporal dynamics of forage and water resources shape space use of West African savanna buffaloes. J Mammal. 92:1287–1297.

Crawford NG. 2010. smogd: software for the measurement of genetic diversity. Mol Ecol Resour. 10:556–557.

Cushman SA, Landguth EL. 2010. Spurious correlations and inference in landscape genetics. Mol Ecol. 19:3592–3602.

Cushman SA, McKelvey KS, Hayden J, Schwartz MK. 2006. Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. Am Nat. 168:486–499.

Epps CW, Palsboll PJ, Wehausen JD, Roderick GK, Ramey RR II, McCullough DR. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecol Lett. 8:1029–1038.

Faubet P, Gaggiotti OE. 2008. A new Bayesian method to identify the environmental factors that influence recent migration. Genetics. 178:1491–1504.

Getz WM, Fortmann-Roe S, Cross PC, Lyons AJ, Ryan SJ, Wilmers CC. 2007. LoCoH: nonparameteric kernel methods for constructing home ranges and utilization distributions. PLoS ONE. 2:e207.

Grobler JP, VanderBank FH. 1996. Genetic diversity and isolation in African buffalo (*Syncerus caffer*). Biochem Syst Ecol. 24:757–761.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Mol Ecol Notes. 2:618–620.

Hedrick PW. 2005. A standardized genetic differentiation measure. Evolution. 59:1633–1638.

Heller R, Okello JB, Siegismund H. 2010. Can small wildlife conservancies maintain genetically stable populations of large mammals? Evidence for increased genetic drift in geographically restricted populations of Cape buffalo in East Africa. Mol Ecol. 19:1324–1334.

Hilty JA, Lidicker WZ Jr, Merenlender AM. 2006. Corridor ecology: the science and practice of linking landscapes for biodiversity conservation. Covelo (CA): Island Press.

Lorenzen ED, Arctander P, Siegismund HR. 2008. High variation and very low differentiation in wide ranging plains zebra (*Equus quagga*): insights from mtDNA and microsatellites. Mol Ecol. 17:2812–2824.

Lowe WH, Allendorf FW. 2010. What can genetics tell us about population connectivity? Mol Ecol. 19:3038–3051.

Lynch M, Ritland K. 1999. Estimation of pairwise relatedness with molecular markers. Genetics. 152:1753–1766.

Martin RB. 2003. Transboundary species report: southern savanna buffalo. Windhoek (Namibia): Ministry of Environment and Tourism.

McRae BH. 2006. Isolation by resistance. Evolution. 60:1551–1561.

McRae BH, Dickson BG, Keitt TH, Shah VB. 2008. Using circuit theory to model connectivity in ecology, evolution, and conservation. Ecology. 89:2712–2724.

Mock KE, Latch EK, Rhodes OE. 2004. Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). Conserv Genet. 5:631–645.

Naidoo R, Du Preez P, Stuart-Hill G, Jago M, Wegmann M. 2012a. Home on the range: factors explaining partial migration of African buffalo in a tropical environment. PLoS ONE. 7:e36527.

Naidoo R, Du Preez P, Stuart-Hill G, Weaver LC, Jago M, Wegmann M. 2012b. Factors affecting intraspecific variation in home range size of a large African herbivore. Landscape Ecol. 27:1523–1534.

Naidoo R, Weaver LC, Stuart-Hill G, Tagg J. 2011. Effect of biodiversity on economic benefits from communal lands in Namibia. J Appl Ecol. 48:310–316.

Nathan R, Perry G, Cronin JT, Strand AE, Cain ML. 2003. Methods for estimating long-distance dispersal. Oikos. 103:261–273.

Peacock MM, Smith AT. 1997. The effect of habitat fragmentation on dispersal patterns, mating behavior, and genetic variation in a pika (*Ochotona princeps*) metapopulation. Oecologia. 112:524–533.

Rousset F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 8:103–106.

Ryan SJ, Knechtel CU, Getz WM. 2006. Range and habitat selection of African buffalo in South Africa. J Wildl Manage. 70:764–776.

Ryman N, Palm S, André C, Carvalho GR, Dahlgren TG, Jorde PE, Laikre L, Larsson LC, Palmé A, Ruzzante DE. 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. Mol Ecol. 15:2031–2045.

Shah VB, McRae BH. 2008. Circuitscape: a tool for landscape ecology. Proceedings of the 7th Python in Science Conference. 7:62–66.

Shirk AJ, Wallin DO, Cushman SA, Rice CG, Warheit KI. 2010. Inferring landscape effects on gene flow: a new model selection framework. Mol Ecol. 19:3603–3619.

Simonsen BT, Siegismund HR, Arctander P. 1998. Population structure of African buffalo inferred from mtDNA sequences and microsatellite loci: high variation but low differentiation. Mol Ecol. 7:225–237.

Sinclair ARE. 1977. The African buffalo: a study of resource limitation of populations. Chicago (IL): University of Chicago Press.

Smouse PE, Long J, Sokal R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool. 35:627–632.

Thomson GR. 2009. Currently important animal disease management issues in sub-Saharan Africa. Onderstepoort J Vet Res. 76:129–134.

Van Hooft WF, Groen AF, Prins HH. 2000. Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa. Mol Ecol. 9:2017–2025.

Van Hooft WF, Groen AF, Prins HH. 2002. Phylogeography of the African buffalo based on mitochondrial and Y-chromosomal loci: Pleistocene origin and population expansion of the Cape buffalo subspecies. Mol Ecol. 11:267–279.

Van Hooft WF, Groen AF, Prins HHT. 2003. Genetic structure of African buffalo herds based on variation at the mitochondrial D-loop and autosomal microsatellite loci: evidence for male-biased gene flow. Conserv Genet. 4:467–477.

Van Hooft WF, Hanotte O, Wenink PW, Groen AF, Sugimoto Y, Prins HH, Teale A. 1999. Applicability of bovine microsatellite markers for population genetic studies on African buffalo (*Syncerus caffer*). Anim Genet. 30:214–220.

Wang JL. 2004. Application of the one-migrant-per-generation rule to conservation and management. Conserv Biol. 18:332–343.

Weir BS, Cockerham CC. 1984. Estimating F statistics for the analysis of population structure. Evolution. 38:1358–1370.

Winnie JA Jr, Cross P, Getz W. 2008. Habitat quality and heterogeneity influence distribution and behavior in African buffalo (*Syncerus caffer*). Ecology. 89:1457–1468.

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