Text S1: Allele size standardization

Microsatellite sets B and C were both available for Masai Mara GR, Lake Nakuru NP and Amboseli NP (total number of genotyped individuals: $N_B = 65$, $N_C = 70$). Microsatellite sets A and B were both available for KNP ($N_A = 459$, $N_B = 38$). Microsatellite sets A and D were both available for KNP and HiP ($N_A = 860$, $N_D = 48$). In the latter comparison, we included both northern and southern KNP, although set D was only analysed in northern KNP, to get more accurate allele frequency estimates for set A. Including both northern and southern KNP was justified because of the very small F_{ST} value between these localities (F_{ST} = 0.0034, 95% CI = 0.0023, 0.0045). In all aforementioned set comparisons, size standardization was based on the allele frequencies of the pooled samples. A detailed depiction of the allele size standardization is given in Table S1. F_{ST} values and G-statistics derived *P* values (9999 randomizations) were estimated with the Genalex add-in for Excel (version 6.503).

Microsatellite sets E and F were only available for one locality each; Serengeti NP and Caprivi Strip, respectively. Standardization of these two sets was only possible by comparison with localities in southern Africa: Niassa Reserve (Niassa analysed with set D, distance to Serengeti NP: ± 1150 km; $N_D = 20$, $N_E = 49$) in case of set E and the pooled samples of northern and southern KNP in case of set F (KNP analysed with set A, distance to Caprivi Strip: ± 1100 km; $N_A = 459$, $N_F = 134$).

Size standardization of sets B and D against set A was unambiguous (Figures 1 and 2), even when allele size difference was ≥ 8 bp (*BM3517* and *INRA006*, Table 1). Allele frequencies in sets B and D strongly correlated with those in set A (*r* per individual locus ≥ 0.88, F_{ST} per individual locus ≤ 0.012). No allele in set B and only one allele in set D (frequency = 0.043) observed more than twice were not observed in set A (more unique alleles vice versa because of the relatively small sample sizes of sets B and D). No significant differentiation was observed between sets, neither for individual loci after Bonferroni correction ($\alpha = 0.0042$, uncorrected $P \geq 0.038$), nor for all loci combined (set B: $P = 0.12$, $F_{ST} = 0.005$, set D: $P = 0.21$, $F_{ST} = 0.004$).

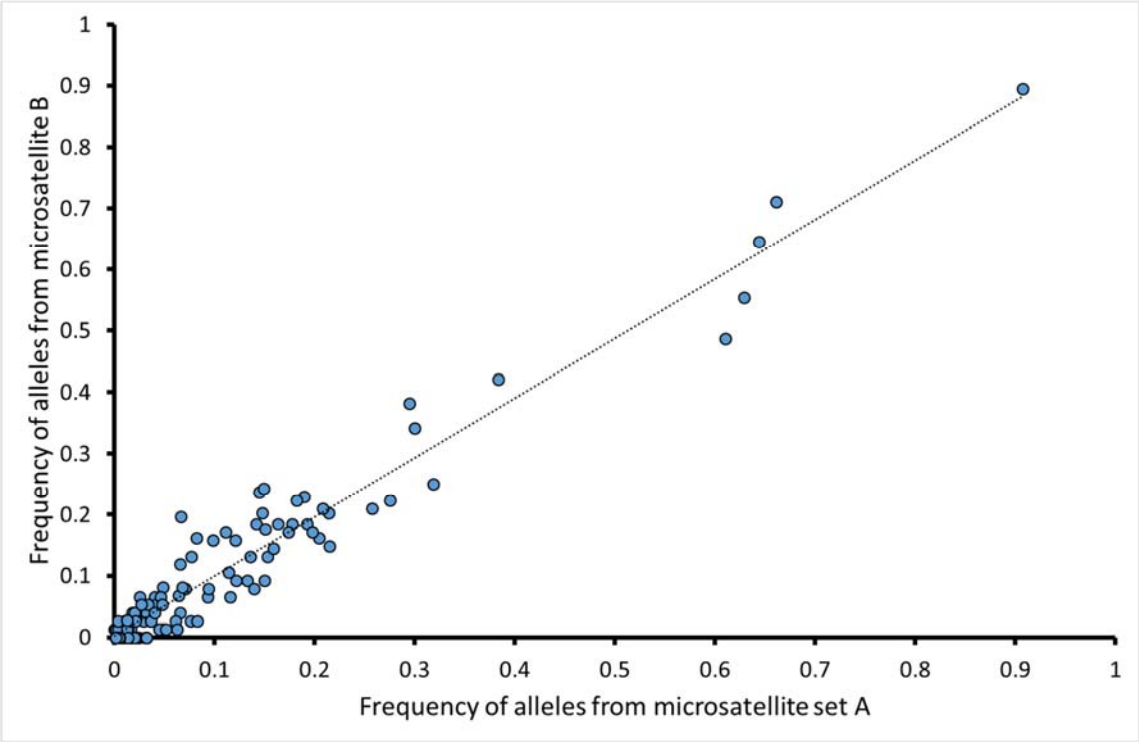

Figure 1: Size standardization of microsatellite set B against microsatellite set A

$N_{microsatellites}$ = 12, $N_{alleles}$ = 122, $N_{individuals}$ = 497, Pearson r = 0.97, F_{ST} = 0.005, P_{FST} = 0.12

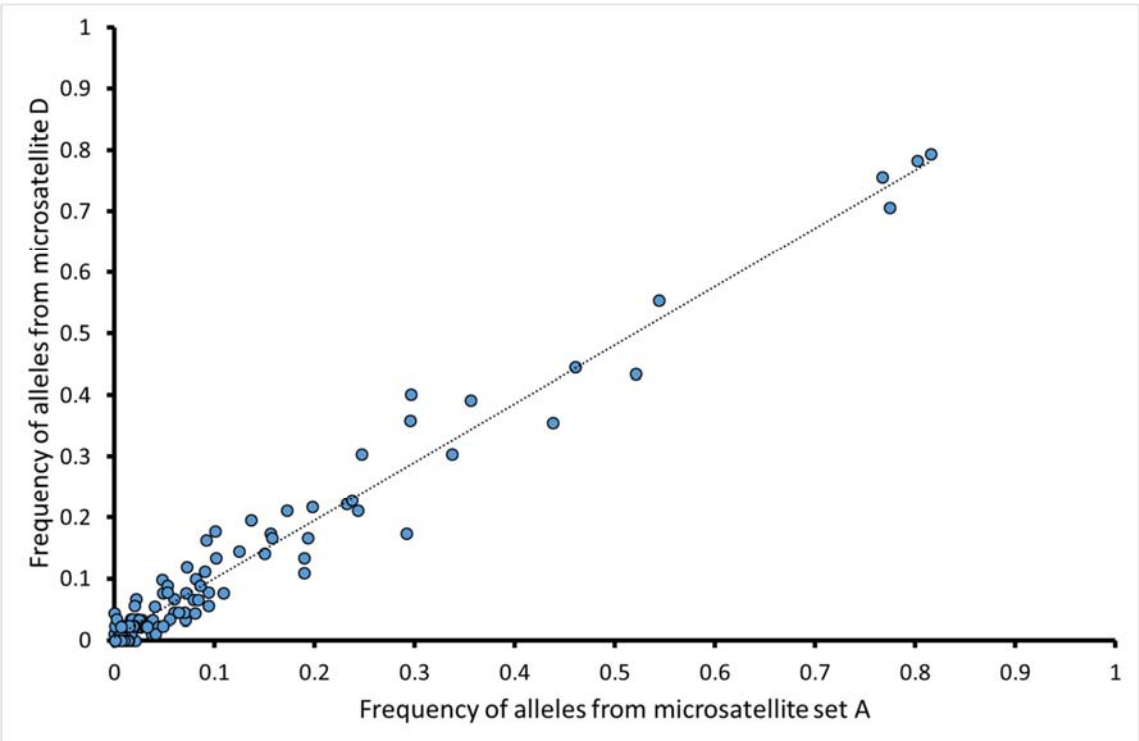

Figure 2: Size standardization of microsatellite set D against microsatellite set A

$N_{microsatellites}$ = 12, $N_{alleles}$ = 108, $N_{individuals}$ = 906, Pearson r = 0.98, F_{ST} = 0.004, P_{FST} = 0.21

Table 1: Size standardization of *BM3517* and *INRA006*, set B against set A

BM3517: $r = 0.998$, $F_{ST} = 0.002$, $P = 0.606$; *INRA006*: $r = 0.956$, $F_{ST} = 0.012$, $P = 0.038$; $N_A = 459$, $N_B = 38$.

BM3517 allele size (A/B)	Frequency set A	Frequency set B
84/92	0.006	0.013
86/94	0.093	0.066
88/-	0.009	0
90/98	0.029	0.026
92/100	0.661	0.711
94/102	0.178	0.184
96/-	0.024	0
INRA006 allele size (A/B)	Frequency set A	Frequency set B
105/-	0.001	0
107/-	0.004	0
109/102	0.149	0.243
111/122	0.062	0.014
113/124	0.610	0.486
115/126	0.148	0.203
117/128	0.012	0.027
119/130	0.012	0.027
127/-	0.001	0

At three loci in set C (*BM0719*, *DIK020* and *ILSTS026*; Table 2), we had to assume that one allele was erroneously called, because its two neighbouring alleles, which differed by two repeats, differed by only one repeat in set B. Erroneous allele calling is far more likely in set C than in set B, considering the aforementioned unambiguous size standardization of set B against set A. At loci *BM0719* and *DIK020*, the erroneously called alleles were observed only once in all the populations analysed with set C (*BM0719*: allele with original size 145 in Nairobi NP, *DIK020*: allele with original size 197 in Laikipia NP). However, the erroneously called allele at locus *ILSTS026* was observed in six out of ten populations at an average frequency of 0.10 (allele with original size 152). This allele could correspond to two alleles in set B; one male-deleterious-trait-associated and the other non-associated. We conservatively chose to let it correspond with the male-deleterious-trait-associated allele because this resulted in a more positive Pearson *r* when correlating allele frequencies against latitude (i.e., less significant allele-frequency cline). Further, allele size calling at locus *ILSTS026* did not influence linkage disequilibrium estimates because neighbouring locus *INRA006* was not included in set C. Despite the relatively high frequencies of the supposedly erroneous called allele, frequencies at locus *ILSTS026* correlated strongly between sets B and C.

Table 2: Size standardization of *BM0719*, *ILSTS026* and *DIK020*, set C against set B

BM0719: $r = 0.874$, $F_{ST} = 0.005$, $P = 0.291$; *ILSTS026*: $r = 0.825$, $F_{ST} = 0.003$, $P = 0.522$; *DIK020*: $r = 0.626$ ($r = 0.861$ without allele 188), $F_{ST} = 0.011$, $P = 0.003$; $N_B = 65$, $N_C = 70$.

BM0719 allele size (A/B/C)	Frequency set B	Frequency set C
132/133/133	0.023	0.014
134/-/135	0	0.007
136/137/137	0.086	0.079
138/139/139	0.023	0.021
140/141/141	0.125	0.093
142/143/143	0.070	0.064
144/145/147	0.125	0.093
146/147/149	0.023	0.021
148/150/151	0.195	0.293
150/152/153	0.023	0.057
152/154/155	0.023	0.029
154/156/157	0.125	0.129
156/158/159	0.156	0.100
ILSTS026 allele size (A/B/C)	Frequency set B	Frequency set C
143/141/144	0.123	0.143
147/145/148	0.200	0.136
149/147/150	0.038	0.029
?/-/152	0	0.014
151/149/154	0.069	0.107
153/152/156	0.146	0.114
155/154/158	0.208	0.186
157/156/160	0.116	0.114
161/160/164	0.069	0.129
163/162/166	0.031	0.029
DIK020 allele size (A/B/C)	Frequency set B	Frequency set C
166/169/169	0.065	0.065
168/171/171	0.016	0.029
170/173/173	0.145	0.232
172/175/175	0.105	0.101
174/177/177	0.040	0.051
176/179/179	0.081	0.109
178/181/181	0.024	0.014
180/-/183	0	0.014
6-7 bp gap in sets B and C		
186/188/189	0.073	0.029
188/190/191	0.218	0.072
190/192/193	0.056	0.130
8 bp gap in sets B and C		
196/-/201	0	0.007
198/200/203	0.073	0.087
200/202/205	0.065	0.036
6 bp gap in sets B and C		
206/208/211	0.032	0.022
28 bp gap in set B		
234/236/-	0.008	0

No significant differentiation was observed between sets B and C when considering all ten loci together ($P = 0.063$, $F_{ST} = 0.005$; Figure 3). Except for locus *DIK020*, no significant differentiation was observed at individual loci after Bonferroni correction ($\alpha = 0.005$, *DIK020*: uncorrected $P = 0.0027$, other loci: uncorrected $P \geq 0.048$). High significance at locus *DIK020* could be attributed to a single allele with a relatively low frequency in set C (frequency set B = 0.22, frequency set C = 0.07; $r = 0.63$ when allele is included, $r = 0.86$ when allele is excluded; Table 2). Because significance could be attributed to a single allele and because F_{ST} was low regardless ($F_{ST} = 0.011$) we do not consider this a meaningful deviation. Except for locus *DIK020*, allele frequencies correlated strongly between sets B and C (r per individual locus ≥ 0.73, F_{ST} per individual locus ≤ 0.007). Only eight alleles (out of 109) observed more than twice in one set were not observed in the other (maximum frequency of these eight alleles = 0.089).

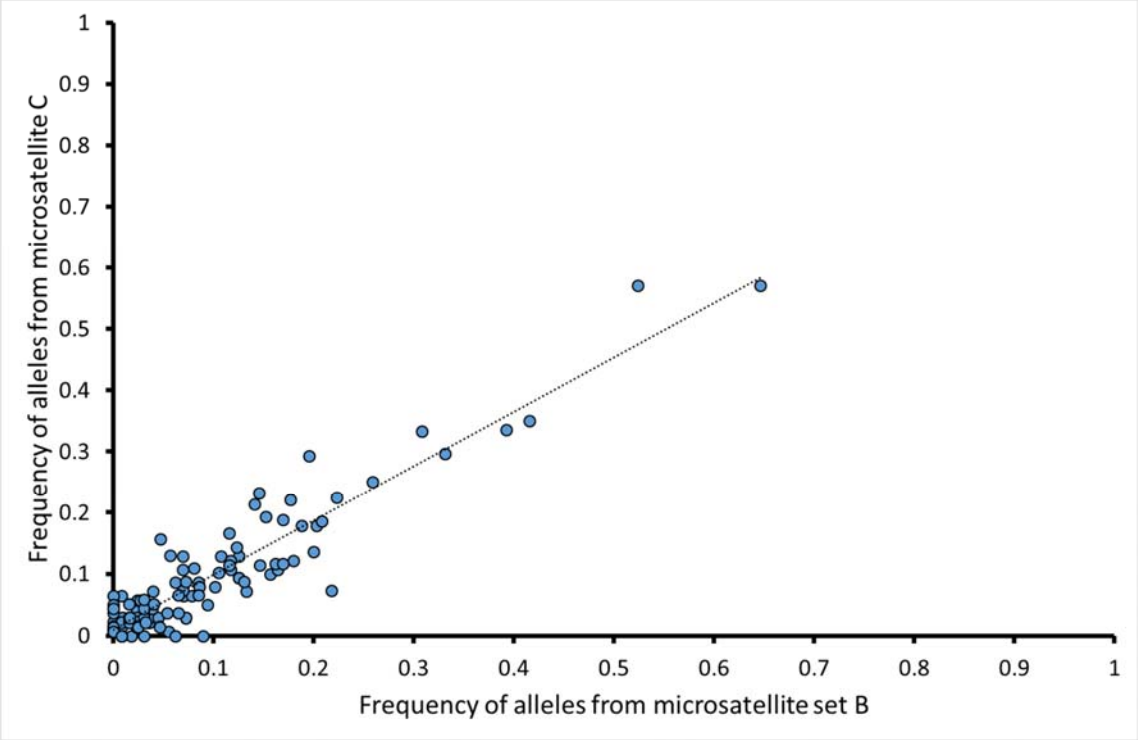

Figure 3: Size standardization of microsatellite set C against microsatellite set B

$N_{microsatellites} = 10$, $N_{alleles} = 109$, $N_{individuals} = 135$, Pearson $r = 0.93$, $F_{ST} = 0.005$, $P_{FST} = 0.063$

Allele frequencies of the three loci in set D were positively correlated with those in set E, with size shifts ≤ 2 bp (*BM1824*: $r = 0.39$, $F_{ST} = 0.031$, $P = 0.003$; *ETH010*: $r = 0.88$, $F_{ST} = 0.058$, $P = 0.0005$; *SPS115*: $r = 0.78$, $F_{ST} = 0.007$, $P = 0.62$; Table 3). Significant differentiation was observed at loci *BM1824* and *ETH010*. However, no significant differentiation was observed at locus *BM1824* when compared with the pooled sample of Mana Pools NP, Nyakasanga, Gorongosa NP and Marromeu GR, which are located 730 km further south ($r = 0.78$, $F_{ST} = 0.012$, $P = 0.065$). Significant differentiation at locus *ETH010* could be attributed due to a unique high-frequency allele in Serengeti NP (frequency = 0.23) not observed in southern Africa (without this allele: $r = 0.92$). The size standardization of the two loci in set F against set A was unambiguous, characterized by high allele-frequency correlations and low F_{ST} values, despite the fact these sets were applied in different populations more than 1000 km apart (*BM3517*: $r = 0.99$, $F_{ST} = 0.004$, $P = 0.023$; *TGLA057*: $r = 0.89$, $F_{ST} = 0.011$, $P < 0.001$; Table 4).

Table 3: Size standardization of *BM0719*, *ILSTS026* and *DIK020*, set E against set D

BM1824: $r = 0.394$ ($r = 0.781$ with the pooled sample of Mana Pools NP, Nyakasanga, Gorongosa NP and Marromeu GR), $F_{ST} = 0.031$, $P = 0.003$; *ETH010*: $r = 0.883$, $F_{ST} = 0.058$, $P = 0.0005$; *SPS115*: $r = 0.781$, $F_{ST} = 0.007$, $P = 0.620$; $N_E = 49$, $N_D = 40$

BM1824 allele size (A/E/D)	Frequency Serengeti NP (set E)	Niassa Reserve (set D)
169/167/169	0.216	0.211 (0.145)
175/175/175	0.136	0.079 (0.092)
177/177/-	0.011	0 (0)
179/179/179	0.011	0 (0.013)
181/181/181	0.273	0.105 (0.250)
183/9183/183	0.091	0.105 (0.039)
185/185/185	0.057	0.026 (.0.066)
187/187/187	0.159	0.053 (0.053)
189/189/189	0.011	0.053 (0.013)
191/191/191	0.023	0.263 (0.066)
193/-/193	0	0.053 (0.066)
195/195/195	0.011	0.026 (0.105)
197/-/197	0	0 (0.066)
199/-/199	0.000	0.026 (0.026)
ETH010 allele size (A/E/D)	Frequency set E	Frequency set D
200/200/-	0.010	0
202/202/-	0.229	0
204/204/204	0.479	0.750
206/206/206	0.271	0.250
208/208/208	0.010	0
SPS115 allele size (A/E/D)	Frequency set E	Frequency set D
223/-/227	0	0.075
227/228/231	0.053	0.025
229/230/233	0.128	0.075
231/232/237	0.117	0.075
233/234/239	0.085	0.100
237/236/-	0.053	0
239/238/245	0.255	0.275
241/240/247	0.064	0.150
243/242/249	0.074	0.100
245/244/251	0.128	0.100
247/246/-	0.043	0
249/-/255	0	0.025

Table 4: Size standardization of *BM3517* and *TGLA057*, set F against set A

BM3517: $r = 0.988$, $F_{ST} = 0.004$, $P = 0.023$; *TGLA057*: $r = 0.887$, $F_{ST} = 0.011$, $P = 0.0001$; $N_A = 459$, $N_F = 134$

BM3517 allele size (A/F)	Frequency KNP (set A)	Frequency Caprivi Strip (set F)
84/-	0.006	0
86/91	0.093	0.112
88/93	0.009	0.011
90/95	0.029	0.078
92/97	0.661	0.690
94/99	0.178	0.108
96/-	0.024	0
TGLA057 allele size (A/F)	Frequency set A	Frequency set B
75/-	0.002	0
79/-	0.001	0
83/-	0.001	0
89/-	0.051	0
91/93	0.001	0.004
93/95	0.275	0.149
95/97	0.257	0.235
97/99	0.295	0.388
99/101	0.076	0.086
101/103	0.040	0.060
103/105	0	0.078