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Effective population size dynamics reveal impacts of historic climatic events and recent anthropogenic pressure in African elephants

J. B. A. OKELLO,*† G. WITTEMYER,*‡§ H. B. RASMUSSEN,‡¶** P. ARCTANDER,** S. NYAKAANA,* I. DOUGLAS-HAMILTON‡ and H. R. SIEGISMUND**

*Molecular Biology Laboratory, Institute of Environment & Natural Resources, Makerere University, PO Box 7298, Kampala, Uganda, †McMaster Ancient DNA Centre, Department of Anthropology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4L9, ‡Save the Elephants, PO Box 54667, Nairobi 00200, Kenya, §Department of Fish Wildlife & Conservation Biology, 115 Wager, Cobrado State University, Fort Collins, CO 80523-1401, USA, ¶Animal Behaviour Research Group, Department of Zoology, University of Oxford, South Park Road, Oxford, OX1 3PS, UK, **Department of Biology, University of Copenhagen, Universitetsparken 15, DK–2100, Copenhagen, Denmark*

Abstract

Two hundred years of elephant hunting for ivory, peaking in 1970–1980s, caused local extirpations and massive population declines across Africa. The resulting genetic impacts on surviving populations have not been studied, despite the importance of understanding the evolutionary repercussions of such human-mediated events on this keystone species. Using Bayesian coalescent-based genetic methods to evaluate time-specific changes in effective population size, we analysed genetic variation in 20 highly polymorphic microsatellite loci from 400 elephants inhabiting the greater Samburu-Laikipia region of northern Kenya. This area experienced a decline of between 80% and 90% in the last few decades when ivory harvesting was rampant. The most significant change in effective population size, however, occurred approximately 2500 years ago during a mid–Holocene period of climatic drying in tropical Africa. Contrary to expectations, detailed analyses of four contemporary age-based cohorts showed that the peak poaching epidemic in the 1970s caused detectable temporary genetic impacts, with genetic diversity rebounding as juveniles surviving the poaching era became reproductively mature. This study demonstrates the importance of climatic history in shaping the distribution and genetic history of a keystone species and highlights the utility of coalescent-based demographic approaches in unravelling ancestral demographic events despite a lack of ancient samples. Unique insights into the genetic signature of mid-Holocene climatic change in Africa and effects of recent poaching pressure on elephants are discussed.

Keywords: African elephants, demographic history, bottleneck, ivory poaching, microsatellite variation, population expansion

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Introduction

Over-harvesting is one of the major drivers of biodiversity loss and species extinction (Caughley 1994; Sala *et al*. 2000; Novacek & Cleland 2001). In addition to direct extirpation, small and isolated populations become susceptible to genetic and stochastic problems associated with small population size (Frankham 1995, 2005). Over the past two centuries, the numbers and range of African elephants declined greatly due to the commercial pursuit for ivory and human population expansion (Cumming *et al*. 1990; Milner-Gulland & Beddington 1993a, b). Such anthropogenic pressure on elephants increased in the 19th century due to expanded international ivory and slave trade (Beachey 1967). Although there were attempts to regulate ivory trade in the early part of the 20th century, poaching driven by the influx of weapons into Africa during the 1970s–1980s severely reduced or extirpated the majority of the continent's

Correspondence: Fax: +1 905 522 5993; jokello@mcmaster.ca; georgew@nature.berkeley.edu

J. B. A. Okello and G. Wittemyer contributed equally to this study.

Table 1 Aerial survey results depicting the number of live and dead elephants counted in the Samburu–Laikipia ecosystem from the 1970s to recent years

Year	Live	Carcasses	Carcass ratio	Count area* (source)
1970	11 500	NA†	NA†	Samburu (Jarman 1973)
1977	1 929	2586	0.57	Samburu (Poole *et al*. 1992)
1985–1987	3 311	613	0.16	Samburu (Poole *et al*. 1992)
1992	2 969	78	0.03	Samburu–Laikipia (Thouless *et al*. 2003)
1999	3 436	92	0.03	Samburu–Laikipia (Kahumbu *et al*. 1999)
2002	5 447	122	0.02	Samburu–Laikipia (Omondi *et al*. 2002)

*Total counts conducted from 1992 to 2002 incorporated the total Samburu–Laikipia ecosystem while sample counts conducted before 1990 were only focused on the Samburu portion of the ecosystem. As a result, a greater area with more elephants was counted in the 1992, 1999, and 2002 counts.
†NA (not available), counts of carcasses were not conducted prior to 1973 in many populations as illegal killing of elephants was rare and not a threat to the persistence of elephant populations.

elephant populations (Douglas-Hamilton 1987; Cumming *et al*. 1990). Concern for the status of the species culminated in the international ban on ivory trade and listing of elephants on Appendix 1 by the United Nations Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1989 (Blanc *et al*. 2003). The rates of killing of elephants subsequently decreased (Stiles 2004). In this study, we explore the genetic ramifications of this human-mediated decline in relation to ancestral demographic signatures in a free-ranging, remnant elephant population in northern Kenya.

Genetic diversity is important to the fitness and long-term survival of species (Saccheri *et al*. 1998; Frankham *et al*. 2002; Frankham 2005). Drastic population size reductions can result in random loss of favourable alleles and fixation of deleterious mutations (Hedrick 2000; Frankham *et al*. 2002). Monitoring of the genetic state of populations often focuses on dynamics in effective population size (N_E, defined as the number of individuals in an idealized population that would show the same effect of random genetic drift as the population under consideration (Wright 1931)). Early detection of N_E reduction is critical for the timely implementation of conservation efforts to avoid possible inbreeding depression and population collapse (Schwartz *et al*. 1998). While the recent demographic history of a population is assumed to be a major determinant of population genetic variation (Frankham 1996), genetic effects of recent human-mediated population declines may be difficult to detect (Luikart *et al*. 1998; Beaumont 1999; Chikhi & Bruford 2005), particularly where major historic demographic events overshadow signatures of contemporary changes (Rogers & Harpending 1992; Storz & Beaumont 2002). Advances in molecular techniques make it possible to infer the variance effective population size from allele frequencies (Waples 1989, 2002; Berthier *et al*. 2002; Beaumont 2003).

We analysed genetic variation and effective population size dynamics of the greater Samburu–Laikipia elephant population of Kenya in order to decipher the history of African savannah elephants, and assess the genetic implications of recent harvesting-mediated population decline. We used a Bayesian coalescence-based approach to estimate past population size changes, based on methods originally developed by Beaumont (1999) and later refined to a hierarchical procedure using multiple loci that are allowed to vary in demographic history. This latter refinement allows for effects of selection or interlocus variation in mutation processes, and aberrant loci are given less weight than in earlier multiplicative, locus-by-locus approaches (Beaumont 1999; Storz & Beaumont 2002). Between 1970 and 1977, about 80% of the elephants in this area were killed and the carcass ratio [$\#Dead/(\#Live + \#Dead)$] counted in 1977 reached an extreme level of 0.57 (Poole *et al*. 1992), with more dead than live elephants observed during aerial surveys (Table 1). Although it is expected that such a marked decline in population size would accelerate the loss of genetic variation and subsequently lower the effective population size (N_E), the impact on genetic diversity may be tempered if N_E remains high (Kalinowski & Waples 2002). We categorized the samples analysed in this study into four age-based cohorts, starting with individuals born in 1960, allowing contemporary analysis over the period of intense poaching. This population serves as a model system for such research in that all individuals are known (Wittemyer 2001) and it is a focal population for African elephant conservation efforts as a MIKE site (Monitoring of Illegal Killing of Elephants) under CITES legislation.

Materials and methods

Sampling and microsatellite analysis

The Samburu elephant population in northern Kenya (37.5 E, 0.5 N) consists of approximately 900 individually known elephants and has been intensively studied and

monitored since 1997 (Wittemyer 2001). It is part of the greater northern population of elephants currently estimated to number 5447 individuals (Blanc *et al*. 2003), comprising the largest elephant populations remaining primarily outside protected areas in the country (Omondi *et al*. 2002). Fresh dung samples (mucosal portions) and a few skin biopsies were obtained from 400 of these elephants and successfully screened for genetic variation at 20 microsatellite loci as described in an earlier study of non–invasive genotyping success, conducted on a 202 individual subsample of the individuals used in this study (Okello *et al*. 2005).

The 400 individuals genotyped in this study represent over 40% of the total Samburu elephant population and 7% of the broader population in the Samburu–Laikipia ecosystem of Kenya. Kenya's elephant populations show weak structure (Okello *et al*. 2008); therefore, results of this study are likely to be representative of a much greater region than Samburu alone. Age estimates of the elephants were conducted using well-established techniques developed on known aged individuals (Moss 1996; Moss 2001). The accuracy of our age estimates was established as ±5 years with 95% confidence when compared to ages derived from molar progression (Laws 1966; Jachmann 1985) assessed during immobilization operations (Rasmussen *et al*. 2005). This, together with the fact that young elephants can be aged more accurately with greater confidence, prompted us to bin the elephants in our study into four age–cohorts (1960–1970, 1971–1981, 1982–1992, and 1993–2003) for analyses. To avoid any possible bias that could arise from sample size differences, we randomly subsampled 80, 79, 80 and 80 individuals, respectively, from each of the four cohorts.

The microsatellite loci analysed were isolated from the African elephant genome and were identified as: LaT05, LaT06, LaT07, LaT08, LaT13, LaT16, LaT17, LaT18, LaT24, LaT25, and LaT26 (Archie *et al*. 2003); FH1, FH39, FH40, H67, FH103 (Comstock *et al*. 2000) ; LA4 and LA6 (Eggert *et al*. 2000); LafMS02 (Nyakaana & Arctander 1998); and LafMS06 (Nyakaana *et al*. 2005). Overall, the study adopted a rigid genotyping process that included a multiple-tube approach and parent–offspring Mendelian checks to confirm the compatibility of alleles. Each individual locus was genotyped at least twice to confirm the genotypes, and for the few inconsistent genotypes, two more repeat genotyping were done with a majority consensus genotype taken (Taberlet *et al*. 1996). This approach yielded high genotyping success with an approximated genotyping error rate of only 2% (Okello *et al*. 2005) and a low estimated average null allele frequency (0.011).

Genetic diversity

Using the program MICRO-CHECKER version 2.2 (Van Oosterhout *et al*. 2004) on the total data set of 400 individuals, we observed no significant evidence of null alleles, short allele dominance, scoring or typographic errors, showing the high reliability of the genotyping procedure adopted. In an earlier study based on the same data set (Okello *et al*. 2005), we found two microsatellite loci showing weak signs of null alleles, as suggested by a general excess of homozygotes (LaT07; $r = 0.067$ and LaT26; $r = 0.079$) but without short or large allele dominance. We did not exclude these two loci from the analysis. Genetic diversity, assessed as average number of alleles, observed and expected heterozygosities, and inbreeding coefficients, was measured across loci and cohorts using the computer program GENETIX version 4.04 (Belkhir *et al*. 2002), which offers the distribution of parameter values by the appropriate resampling scheme of the relevant objects. Exact tests for deviations from Hardy–Weinberg proportions at each locus were assessed for both the total and cohort subsamples. Tests for genotypic linkage disequilibrium for each pair of loci in all cohorts were conducted using the program GENEPOP version 3.4 (Raymond & Rousset 1995b). Exact tests for population differentiation among cohort subsamples (Raymond & Rousset 1995a) were also conducted using GENEPOP, taking them as different populations. Departures from Hardy–Weinberg proportions in cohorts were assessed by unbiased exact tests and unbiased probability (p) values estimated through a Markov chain method with 10 000 dememorization steps, 1000 batches, and 5000 iterations per batch (Rousset & Raymond 1995). To minimize effects of type I error in case of multiple comparisons, we performed sequential Bonferroni corrections (Rice 1989; Holm 1979) on P values for each cohort and the overall sample.

Effective population size dynamics

To assess changes in effective population size and date the timing of these changes, we used the hierarchical Bayesian Markov Chain Monte Carlo (MCMC) simulation model implemented in the computer program MSVAR version 1.3 (Storz & Beaumont 2002). This method is based on a stepwise mutation model and is used to assess the magnitude and timing of the most recent, major demographic change event. Prior distributions of ancestral (N_0), current (N_1) effective population sizes, time since the change in effective population size began (T), and the mutation rate (θ) are assumed to be log normal, and the means and standard deviations of these distributions are themselves drawn from prior distributions with the latter truncated at zero. We conducted five independent runs on the total data set as well as on each of the four age-based cohort subsamples, using uninformed priors with large variances (details in Table S1, Supplementary material), to minimize their effects on posterior distributions. Hyperpriors of the first two runs assumed N_1 to be larger than N_0 and those of the last three runs assumed N_1 and N_0 to be of the same size (no population size changes). The k

and *g* tests for population expansion (Reich & Goldstein 1998; Reich *et al*. 1999) and homozygosity (or its complement heterozygosity) excess tests (Cornuet & Luikart 1996) were also implemented on the study data set as detailed in the Supplementary material.

To evaluate the impact of demographic model structure on the posterior results of MSVAR, we compared results from five independent runs on the total data set using either a linear or exponential demographic model (Fig. S1, Supplementary material). Because the exponential demographic model is expected to be more accurate than the linear model for modelling recent population declines (Beaumont 1999), we assumed results using the exponential model are the most salient to the question posited in this study regarding the impacts of over–harvesting on N_E. The generation time for the Samburu elephant population has not been estimated; therefore we used the generation time of 17.4 years from the Amboseli elephant population in southern Kenya (Moss 2001), a nearby population with similar ecology and social structure, in calculations of the time since the decline began (T). To assess the degree to which T was dependent on this model parameter, we conducted five independent runs using an upper generation time of 25 years (Fig. S1). Finally, to ensure that the effects of other factors such as population substructure are not influencing results, we analysed another subset data consisting of 100 samples from seven distinct elephant populations across Kenya.

Overall, each of the five independent chains per data set were simulated for up to 2×10^9 MCMC iterations, recording parameter values for every set of 1×10^5 iterations to give 20 000 recorded parameter sets from the posterior distribution. We discarded the first 10% of recorded values for each chain (when simulations may not have stabilized), and processed the data using the computer program BOA version 1.1.4 (Smith 2005) for R version 2.3.1 (R-CDT 2006). The Brooks, Gelman and Rubin Convergence diagnostic tests were done using BOA on all the data chains to check statistically for convergence (Gelman & Rubin 1992; Brooks & Gelman 1998). Convergence of the chains is demonstrated where the corrected scale reduction factor output approximates a value of 1, indicating the samples have arisen from a stationary distribution (Smith 2005). The potential scale reduction factors for all three parameters were approximately 1 ($\mathrm{Log}_{10}N_0 = 1.0$; $\mathrm{Log}_{10}N_1 = 1.0$; $\mathrm{Log}_{10}T = 1.2$), providing statistical evidence for convergence of the chains. Thereafter, the last 50% of the data from the five chains were combined (50 000 sample points) and summary statistics of the marginal posterior distributions of $\mathrm{Log}_{10}N_0$, $\mathrm{Log}_{10}N_1$, and $\mathrm{Log}_{10}T$ were estimated as the mean, 0.025 and 0.975 quantiles. The ratio (r) of the posterior distributions of current and ancestral effective population sizes (where $r = N_0/N_1$) were calculated to ascertain the dynamics of the elephant population, where $r = 1$ indicates stability, $r > 1$ indicates expansion, and $r < 1$ indicates decline in the effective population size (Beaumont 1999). The density distribution of time in years since the population started declining as a function of the MCMC state for each of the four age-based cohorts were estimated using the computer program TRACER version 1.4 (Rambaut & Drummond 2007).

In order to assess for the possible influence of gene flow on reported effective population size changes, we performed self-classification assignment analysis to identify possible first-generation migrants using the computer program GENECLASS version 2 (Piry *et al*. 2004). This program can assign individuals probabilistically to their candidate populations by their multilocus genotype, and is also capable of identifying individuals not belonging to a population in question (i.e. likely migrants). Self-classification runs were performed on the total sample using the Bayesian individual assignment method (Rannala & Mountain 1997) to estimate the likelihood that an individual elephant originated from the study population. The marginal probability of a given multilocus genotype was compared to those generated from 10 000 Monte Carlo random resamplings (Paetkau *et al*. 2004), where an individual with a $P < 0.05$ is assumed not to belong to the population, and hence, a first-generation immigrant. In addition, we compare results from analyses of multiple populations and different age-dependent cohorts.

Results

Genetic diversity

Levels of genetic diversity, measured in terms of average number of alleles, expected and observed heterozygosities were generally high in the study population, but varied slightly across four age–based cohorts (Table 2). Average number of alleles with correction for possible differences in sample sizes was lowest (8.95) in the 1971–1981 cohort and highest (9.45) in the 1960–1970 cohort, whereas the mean expected heterozygosity was similar among cohorts.

The overall fixation indices were low but positive in all cohorts, with three of the cohorts showing significant deviations from Hardy–Weinberg proportions: 1960–1970 ($F_{IS} = 0.025$; $P_{HW} = 0.002$), 1982–1992 ($F_{IS} = 0.070$; $P_{HW} < 0.001$), and 1993–2003 ($F_{IS} = 0.060$; $P_{HW} = 0.003$) but not the 1971–1981 cohort ($F_{IS} = 0.004$; $P_{HW} = 0.196$). Likewise, less than 5% of loci (four of 80 compared; 20 microsatellite loci analysed in each of the four cohorts) showed evidence of significant deviation from Hardy–Weinberg proportions after sequential Bonferroni corrections, conducted for each cohort independently. These significantly deviated loci occurred in 1982–1992 and 1993–2003 cohorts, with no loci demonstrating significant deviation across all cohorts analysed (Table 2). No evidence was found of short allele dominance or large allele dropouts at any of the 20 loci, characteristics suggestive of significant effects of null alleles.

Table 2 Genetic diversity in the Samburu elephant population. Diversity was measured in terms of number of alleles (A), expected (H_E) and observed (H_O) heterozygosities, probability of deviation from Hardy–Weinberg proportions $P_{(HW)}$ and an analogue of Wright's fixation index, F_{IS} (Hartl & Clark 1997) for the microsatellite loci analysed in the four temporal samples. Significant P values for HW after sequential Bonferroni corrections are in bold

	1960–1970 (N = 80)					1971–1981 (N = 79)					1982–1992 (N = 80)					1993–2003 (N = 80)				
Locus	A	H_E	H_O	P_{HW}	F_{IS}	A	H_E	H_O	P_{HW}	F_{IS}	A	H_E	H_O	P_{HW}	F_{IS}	A	H_E	H_O	P_{HW}	F_{IS}
LaT05	13	0.86	0.86	0.180	0.004	11	0.87	0.93	0.584	–0.071	13	0.85	0.86	0.004	0.115	12	0.84	0.75	0.251	0.111
LaT06	17	0.73	0.71	0.139	0.040	16	0.67	0.73	0.911	–0.070	20	0.71	0.67	0.061	0.062	18	0.76	0.77	0.498	–0.012
LaT07	17	0.89	0.83	0.364	0.065	17	0.89	0.84	0.302	0.060	18	0.91	0.67	**< 0.001**	0.267	18	0.90	0.77	0.046	0.145
LaT08	11	0.83	0.86	0.363	–0.036	14	0.84	0.89	0.810	0.044	12	0.82	0.77	0.203	0.059	11	0.79	0.71	0.139	0.114
LaT13	11	0.77	0.70	0.467	0.099	9	0.80	0.75	0.669	0.071	8	0.77	0.74	0.155	0.048	7	0.77	0.74	0.894	0.048
LaT16	11	0.78	0.72	0.060	0.088	8	0.78	0.74	0.786	0.051	8	0.76	0.64	0.070	0.169	8	0.79	0.73	0.169	0.077
LaT17	12	0.84	0.80	0.234	0.060	10	0.82	0.76	0.024	0.088	11	0.82	0.69	0.029	0.160	11	0.82	0.76	0.644	0.071
LaT18	10	0.83	0.80	0.144	0.038	10	0.79	0.76	0.092	0.049	10	0.81	0.67	0.056	0.178	9	0.81	0.78	0.389	0.042
LaT24	11	0.85	0.86	0.132	–0.004	10	0.83	0.82	0.815	0.013	10	0.93	0.80	0.377	0.043	10	0.86	0.81	0.756	0.062
LaT25	9	0.83	0.78	0.250	0.074	8	0.83	0.80	0.710	0.044	9	0.80	0.75	0.072	0.074	9	0.84	0.83	0.210	0.027
LaT26	12	0.86	0.78	0.109	0.100	12	0.87	0.78	0.021	0.111	11	0.83	0.65	**< 0.001**	0.219	13	0.84	0.66	**0.001**	0.224
FH1	5	0.59	0.65	0.274	–0.097	5	0.62	0.73	0.421	–0.186	5	0.59	0.66	0.023	–0.112	5	0.58	0.85	0.394	0.120
FH39	10	0.76	0.66	0.014	0.133	10	0.76	0.82	0.342	–0.073	10	0.77	0.85	0.516	–0.010	10	0.78	0.70	0.579	0.106
FH40	6	0.55	0.50	0.019	0.090	5	0.58	0.56	0.218	0.041	6	0.49	0.51	0.395	–0.032	5	0.47	0.51	0.688	–0.089
FH67	9	0.71	0.91	0.568	0.004	7	0.68	0.72	0.154	–0.056	8	0.66	0.78	0.367	–0.167	8	0.72	0.70	0.554	0.029
FH103	5	0.47	0.52	0.581	–0.092	5	0.58	0.52	0.016	0.115	5	0.59	0.54	0.125	0.100	5	0.52	0.59	0.379	–0.127
LA4	5	0.65	0.63	0.436	0.045	5	0.67	0.68	0.892	–0.021	5	0.63	0.59	0.053	–0.071	5	0.63	0.69	0.260	–0.084
LA6	5	0.54	0.68	0.173	–0.240	4	0.52	0.51	0.953	0.025	3	0.51	0.45	0.211	0.121	5	0.52	0.34	**< 0.001**	0.363
MS02	5	0.70	0.71	0.024	–0.010	5	0.69	0.73	0.746	–0.051	6	0.67	0.76	0.489	–0.127	5	0.72	0.74	0.507	–0.038
MS06	6	0.68	0.70	0.637	–0.021	8	0.71	0.76	0.567	–0.060	8	0.63	0.66	0.826	–0.039	7	0.63	0.64	0.586	–0.008
Overall	9.5	0.74	0.72	**0.002**	0.025	9	0.74	0.74	0.196	0.004	9.3	0.72	0.68	**< 0.001**	0.070	9.1	0.73	0.69	**0.003**	0.060

Table 3 Current and ancestral effective population sizes and time since the onset of decline for the Samburu total population and each of four age-based cohorts, and the sample of the overall Kenyan population (containing individuals randomly sampled from seven Kenya populations). These estimates were based on hierarchical Bayesian MCMC simulations using the exponential assumption of population changes. Bracketed are their 0.025 and 0.975 quantiles

Sample	Size	Current N_E (N_0)	Ancestral N_E (N_1)	Time in years (T)
Samburu total	319	647 (180–2259)	4 805 (1347–17 204)	2465 (523–10 994)
1960–1970	80	942 (83–6485)	4 932 (1380–17 311)	2516 (114–66 322)
1971–1981	79	628 (14–111 198)	3 471 (919–12 502)	356 (0.16–9615)
1982–1992	80	666 (65–4720)	4 931 (1395–17 923)	2543 (97–82 647)
1993–2003	80	890 (179–3941)	4 319 (1174–16 135)	2595 (174–21 092)
Overall Kenya	100	641 (141–2999)	103 039 (27 699–393 550)	7161 (1309–33 574)

Therefore, population substructure resulting from female philopatry among elephants was the probable driver of these weak signals.

Fisher's methods of combining test probabilities across all samples revealed no significant pairwise linkage disequilibrium after sequential Bonferroni corrections. Significant genetic differentiation was observed between the 1971–1981 and 1993–2003 ($\chi^2 = 72.91$, d.f. = 40; $P = 0.001$) and to a lesser extent the 1960–1970 and 1971–1981 cohorts ($\chi^2 = 61.73$, d.f. = 40; $P = 0.015$; although not significant after Bonferroni correction of α levels). The overall differentiation among the four cohorts was not significant at an $\alpha = 0.05$ level ($\chi^2 = 54.67$, d.f. = 40; $P = 0.061$).

Effective population size dynamics

The results of the MSVAR procedure for assessing and dating population size changes identified the most recent population demographic event as a decline from an estimated ancestral effective population size (N_1) of 4805 (1347–17 204) to a current one (N_0) of 647 (180–2259). The estimated time since this decline started was 2465 years (523–10 994), based on the more realistic elephant generation time of 17.4 years (Moss 2001; see Table 3 for details). Results were similar regardless of the demographic model applied (linear or exponential) or the generation time used in the analysis, although increasing the generation time or applying the linear model somewhat increased the estimated time since decline (Fig. 1). Across all runs, the combined maximum ratio r (= N_0/N_1) of current and ancestral effective population sizes was 0.491, with a mean value of 0.13, thus providing clear support for population decline rather than stability or expansion (Fig. 1D). Kernel density plots for N_0, N_1 and T, were relatively smooth and unimodal, with no significant variations across the five independent chains simulated for each analysis (Fig. S1).

Independent analyses of each of the four age-based cohorts indicated that a distinct genetic event impacted the 1971–1981 cohort, substantiating the previously presented results of genetic differentiation tests and F_{IS} for this cohort. Although the posterior distributions of N_E were broad, the lowest mean and 95% interquantile range was found for the 1971–1981 cohort. For the three other cohorts, the 95% interquantile posterior density distribution results for T did not include the last 100 years, whereas for the 1971–1981 cohort, the tail (containing over 10% of simulation results) were skewed towards the most recent decade, providing evidence of very recent population decline during the period when individuals in this cohort were sired in addition to the older Holocene-dated decline that was consistent across all cohort and total samples (Fig. 2, Table 3). Results from BOTTLENECK provide no evidence of a bottleneck, as expected, considering that the post-decline population effective population size is estimated at over 600 individuals. Further analysis of effective population size changes using the k and g tests and homozygosity excess tests were inconclusive (Supplementary material). Finally, we detected no evidence of any first-generation immigrant based on a self-classification analysis performed on the total sample using the Bayesian individual assignment method in GENECLASS ($P < 0.05$), thereby ruling out first-generation immigrants as the likely cause of the expansion signature observed above.

Discussion

Genetic diversity

Genetic diversity indices in terms of the mean number of alleles, expected and observed heterozygosities were high (Table 2) compared to values of 0.19, 0.18, 0.19 for Addo National Park in South Africa (Whitehouse & Harley 2001) or 7.25, 0.72, 0.77 for Queen Elizabeth National Park in Uganda (Muwanika *et al.* 2003). The much lower genetic diversity in Addo elephant population has been attributed to a recent population bottleneck that resulted from a founder

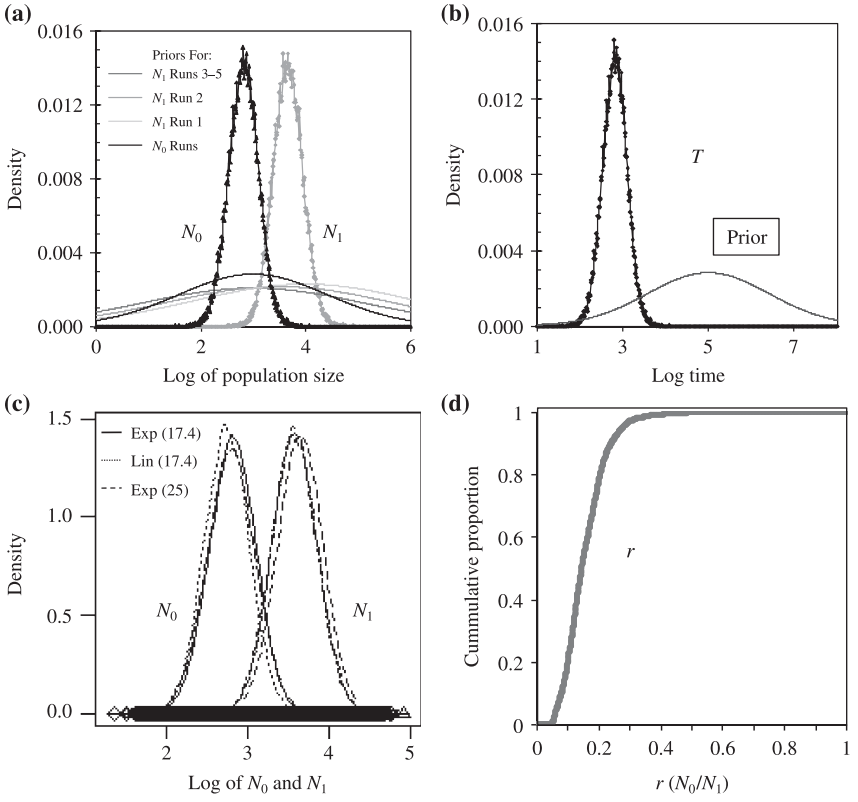

Fig. 1 The posterior distributions of (A) the current (N_0) and ancestral (N_1) effective population sizes and (B) the time since the onset of decline in logarithmic scales for the total sample assuming exponential demographic change and a 17.4-year generation time. (C) The decline indicated by posterior distributions of N_0 and N_1 was consistent in magnitude across three different model parameterizations, being Lin (17.4), the linear change demographic model with a generation time of 17.4 years; Exp (17.4), the exponential change demographic model with a generation time of 17.4 years; and Exp (25), the exponential change demographic model with a generation time of 25 years. (D) The cumulative graph of the ratio of N_0/N_1 (r) across MCMC simulation states clearly demonstrates demographic decline with r always below 0.5. In a growing population, r would be distributed above 1 while in a stable population r would be distributed around 1.

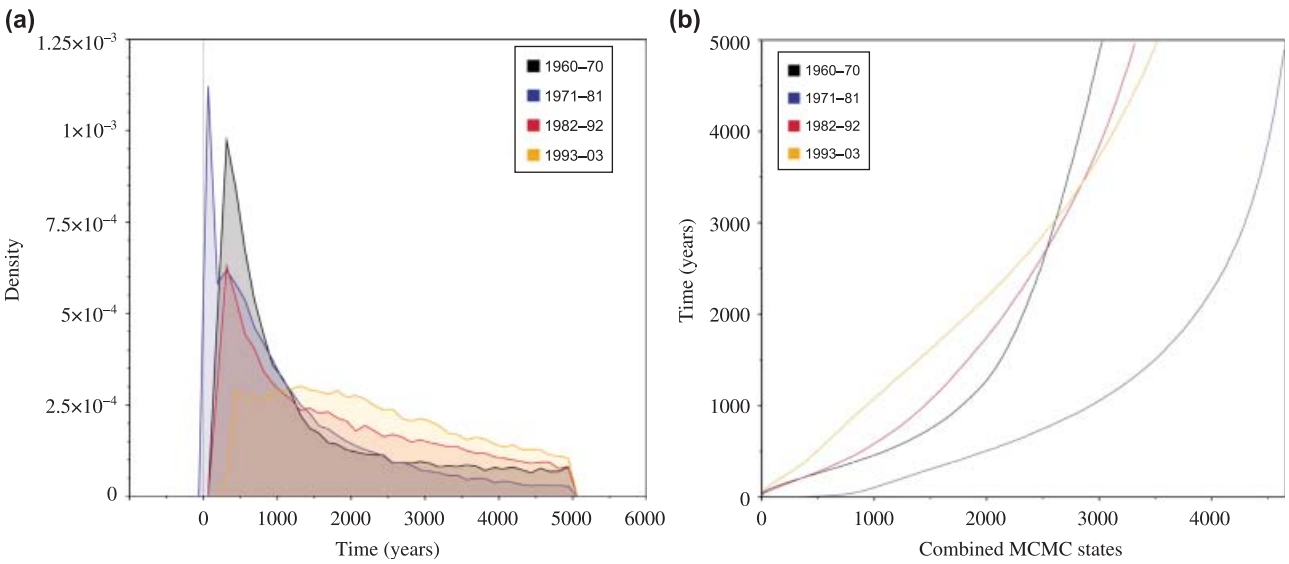

Fig. 2 Comparison of the distribution of estimated time since the onset of decline (natural scales) obtained from MSVAR among the four age-based cohorts (A), and MCMC trace for the last 5000 years (sorted in ascending order) depicting differences in the weight of temporal estimates of the onset of decline generated from TRACER (B). Both graphs demonstrate that the 1971–1981 cohort behaves differently, its time estimate skewed to recent years relative to the other cohorts, an attribute of anthropogenic pressure that occurred when individuals in this cohort were born.

population of 11 individuals reintroduced after the near extermination of this elephant population in the year 1920 (Hoffman 1993). While the Queen Elizabeth elephant population showed a slightly lower genetic diversity than the study population, the difference between the two populations is minimal in comparison to the extreme low levels of genetic diversity in Addo. Like Samburu, heavy poaching for ivory drastically reduced the Queen Elizabeth

population in the 1970–1980s (Parker & Douglas-Hamilton 1976), and the genetic bottleneck observed in that population (Muwanika *et al*. 2003) was attributed to the recent human-mediated population reduction. The concurrent poaching-related population decline that occurred in the Samburu elephant population, however, only resulted in a short- but not long-term detectable genetic signal as discussed below.

Although allelic diversity is one of the most sensitive measures for inferring population bottlenecks (Leberg 1992; Spencer *et al*. 2000), it only varied slightly across the cohorts. The extent of population size decline, its duration and the rate of recovery are all important factors that determine the ability of a population to recover its heterozygosity at neutral loci such as microsatellites (Nei *et al*. 1975). Diversity loss may occur when population sizes decline to very small levels and become more pronounced if the decline is prolonged (Motro & Thomson 1982). Recent excessive poaching pressure in the study elephants covered less than one generation followed by a period of sustained population growth, and hence no detectable loss in number of alleles was observed. This is not surprising since the expected loss of diversity per generation is $1/(2*N_E)$; where N_E is the effective population size. In the case of a much skewed sex ratio among breeding females and males with the number of females being much higher than the number of males, N_E approaches four times the number of breeding males. Therefore, the expected loss would probably be less than $1/(8*N_M)$; where N_M is the number of reproducing males in the Samburu area. This value is too small to be detected.

Genetic signal of recent poaching

The poaching of elephants for their ivory in the study area was extreme (Table 1) with more dead than live elephants observed in a 1977 survey (Poole *et al*. 1992), a conservative estimate of poaching pressure considering aerial carcass counts are low in comparison to actual ground death registration (Parker & Douglas-Hamilton 1976). Although contemporary population size fluctuations can reduce genetic diversity within a population (Motro & Thomson 1982) leading to low values of N_E compared to census sizes (Piry *et al*. 1999), the elephant population size in the present study appears to have remained above the critical N_E level of 500 individuals to maintain long-term evolutionary potential (Franklin & Frankham 1998; Lynch & Lande 1998) during its recent poaching-mediated decline. Corroborating the above census trend, however, our assessment of recent genetic signatures of population changes revealed interesting results for the cohort sired during the poaching epidemic of the 1970s. Inbreeding coefficient (F_{IS}) values declined between the 1960–1970 and 1971–1981 cohorts to nearly zero followed by increased values in the 1982–1992 and 1993–2003 cohorts (Table 2). The overall F_{IS} were positive in all cohorts, potentially caused by population social structure and nonrandom mating resulting from elephant behavioural characteristics of female philopatry and male-biased dispersal (Nyakaana & Arctander 1999) and reproductive monopolization by older males (Rasmussen *et al*. 2008). Similarly, results from Bayesian-based MCMC simulations implemented in MSVAR (Beaumont 1999; Storz & Beaumont 2002) for each cohort show an N_E reduction in the cohort of elephants sired between 1971 and 1981 (when extreme poaching rates occurred) relative to the other three cohorts for which N_E estimates remained relatively similar, although the distributions of these values overlap (Table 3).

Additionally, estimates of the timing of population size decline from this program indicate that over 10% occurred in the last decade for the 1971–1981 cohort , in stark contrast to the other three cohorts which show little evidence of decline in the last century. These differences registered in the 1971–1981 cohort are more surprising when considering that probable errors in age estimates are likely to weaken any demographic signal ascribed to a specific age cohort. Including individuals born prior to the poaching peak (1973–1977) in the cohort is likely to dilute any signal related to poaching. Similarly, if these results are driven by behavioural modification in relation to poaching, including elephants born after the poaching period is likely to weaken signals. While differences were found in recent decades, the posterior distributions of all four cohorts indicate the period of major decline occurred approximately 2500 years ago.

Ivory poaching selectively removes the large, primary breeding male elephants and social group matriarchs which carry the largest tusks (Eltringham & Malpas 1980; Hall-Martin 1980), thereby impacting demographic processes and social organization. In a population where approximately 80% were removed in less than a decade, it is likely that major social disruptions occurred as a consequence of poaching pressure. The dynamics in N_E and F_{IS} values across the contemporary period potentially reflect an increase in reproductive skew resulting from the removal of the majority of breeding males during the poaching-impacted 1971–1981 cohort and social behaviour breakdown resulting from the killing of matriarchs as recorded in other poached populations (Nyakaana *et al*. 2001). However, as younger individuals sired prior to the poaching matured and parented calves in the latter two cohorts (post-1980), reproductive skew likely declined and the original genetic diversity carried across in young individuals was again infused into the new generations. Additionally, higher degrees of social stability among the post–poaching era elephants potentially drive the increase in F_{IS}, which in this case may reflect the resumption of natal philopatry as social organization becomes restored. Thus, reduced N_E and F_{IS} values in the 1971–1981 cohort were only temporary, with MSVAR cohort results for pre-1970 and post-1981 cohorts being similar (Table 3).

Population changes in relation to climatic history

While a transient signal of contemporary reduction in the effective population size was found in the cohort sired during the 1970s, results from analyses in MSVAR demonstrate a more drastic decline dated to about 2500 years ago. The estimated current effective number of elephants based on the Samburu population alone is approximately 650, compared to an ancestral size of approximately 5000 individuals. Results from analysis of samples representing seven populations across Kenya (see Okello *et al*. 2008 for details) were similar, with the ancestral size inferred at approximately 100 000 individuals, and the current population and time since decline falling within the same confidence intervals although the mean time since decline was greater (Table 3). The similarity of these results indicates that the effective population size changes attributed to the Samburu population are representative of a much greater area. At a minimum, the elephants of Kenya exhibit the same signatures, although it is likely these signatures represent most of East Africa and perhaps beyond. The estimated time since decline approximately coincides with a major drying trend that began in sub-Saharan Africa about 4000 years ago during which multiple, long-term droughts lasting up to 300 years impacted the study region (Hassan 1997; Cullen *et al*. 2000; Thompson *et al*. 2002). Such a drying trend likely reduced the range available to elephants (Surovell *et al*. 2005), and potentially drove a severe decline in African Savannah elephant populations during the mid- Holocene. We therefore interpret the genetic signature in the study elephants as the result of population decline driven by drying climatic conditions and desert expansion that dominated the last half of the Holocene in tropical Africa. This signature of a historic decline has also been observed in African buffalos based on Bayesian-coalescent analysis (Heller *et al*. in review), indicating mid-Holocene climatic cycles affected other large African mammals as well.

In addition to the hierarchical Bayesian method, the *k* and *g* tests for population expansion (Reich & Goldstein 1998; Reich *et al*. 1999) and homozygosity (or its complement, heterozygosity) excess tests that test for deviations from the mutation-drift equilibrium (Cornuet & Luikart 1996), were also applied to assess population change as detailed in the Supplementary material. Results from analyses using BOTTLENECK and the *k* and *g* tests did not provide evidence of a bottleneck in the demographic history of the study population (Tables S2 and S3). Although the homozygosity excess that was shown may indicate an expansion, we think it is merely an artefact, due to the sensitivity of the above tests to other factors such as deviation from Hardy–Weinberg proportions or socially mediated substructure characteristic of elephant populations. In contrast, the timing and magnitude of decline registered through the Bayesian analysis implemented in MSVAR appeared to be robust to such influences, as results were consistent in the total and for each of the four age-based cohorts as well as for the analysis of samples from the seven populations across Kenya.

The elephant demographic history detected in this study were derived by assuming a stepwise mutation model for the hierarchical Bayesian method and an enclosed population. Mutations sometimes occur by multistep changes in allele size and this could create gaps in the distribution of allele sizes and produce a pattern of variation similar to that generated by a population bottleneck (Storz & Beaumont 2002). Similarly, immigration of different alleles from another population could create such gaps if the immigrant alleles are outside the range of allele sizes of the recipient population (Garza & Williamson 2001). However, multistep changes appear to be very infrequent relative to single-step changes, and hence, this would potentially affect only a small proportion of microsatellites (Storz & Beaumont 2002). Nonetheless, any significant bias would be downplayed by using a large number of microsatellites, as in this study, and hence the inferred demographic signal is largely inherent in the data. With regard to potential immigrant alleles, the Bayesian self-classification analyses showed no potential presence of first-generation immigrants, demonstrating that deviations from mutation–drift equilibrium observed are unlikely to be caused by immigrants. As a function of human population growth and accompanying habitat modification, elephant populations are becoming more isolated. Reduction in effective population size due to human-induced isolation is likely to result, a potential confounding influence of effective population change if the Samburu population has undergone recent isolation. While the primary human population growth and land use changes have occurred in the last half century, radio-tracking information indicates that the greater Samburu–Laikipia ecosystem remains well-connected (unpublished results) and our analysis of young individuals (elephants born between 1992 and 2002) did not differ from those of the oldest cohort (elephants born prior to 1971). Furthermore, the signals presented here are found for an analysis of the greater Kenyan elephant population. As such, increasing human pressure driving isolation is not likely to drive the dynamics in effective population size reported here, although this may be a salient factor in other populations.

Management and conservation implications

While genetic evidence of the recent effects of anthropogenic poaching was found, allelic diversity was not detectably affected, on account of the short duration of the poaching epidemic. Rather it appears to have been only temporarily impacted and able to rebound as elephants sired prior to the epidemic became reproductively active. As populations declined and targets became more elusive, poaching moved

on to higher density areas, allowing genetic diversity to be retained by remnant elephants. It is likely that drastically different genetic impacts would have resulted if harvesting had continued for the duration of an entire generation. Because this analysis was conducted on a free-ranging wild elephant population, assumptions of the analytical techniques used were not always strictly upheld. The robustness of our results across different age-cohorts in the population and in relation to techniques analysing different features of genetic variability while relying on the same mutation model, however, indicate our interpretation of the genetic signatures found are likely a feature of actual demographic changes of the study population.

In light of the results from this study, populations documented to have experienced effective population size dynamics interpreted as resulting from recent events may actually be showing ancestral signatures. At present, deciphering time-specific changes in effective population size dynamics can be conducted using multiple analytical approaches that pick information at different evolutionary times. Development of analytical methods designed specifically to decipher complex demographic histories such as that found in African elephants, however, are needed. As demonstrated in this study, the investigation of genetic signatures of ancient population trends without historic samples using coalescent-based modelling and analysis of allele frequency can offer important insight into the evolutionary history of other species.

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John B.A. Okello is interested in the origins, diversification, and population size changes of African flora and fauna, and evolutionary interplay of infectious diseases affecting the world's human and wildlife populations. George Wittemyer studies the conservation, ecology and behaviour of wildlife. Henrik B. Rasmussen works on the conservation of wildlife end wilderness areas in Africa. Silvester Nyakaana's interest include population, evolutionary and conservation genetics of large mammals. Peter Arctander works on molecular evolution of biological complexity, focusing on evolution of regulatory systems and information handling in eukaryote organisms. Iain Douglas-Hamilton has spent more than 40 years working on the conservation of elephants in Africa and currently runs an organization focusing on that aim. Hans R. Siegismund works on the evolutionary interplay of foot-and-mouth disease in the wildlife-livestock-disease interface in East Africa.

Supplementary material

The following supplementary material is available for this article:

Supplementary Information. Effective population size dynamics reveal impacts of historic climatic events and recent anthropogenic pressure in African elephants

Fig. S1 The independence of results to model parameters as demonstrated by the consistency of the chains across each of the five runs and compiled results across analyses *r* using different generation times and either the exponential or linear demographic model.

Table S1 Priors of model parameters used to simulate five independent chains of MSVAR runs

Table S2 Population expansion analyses in African elephant based on the *k* and *g* test of 10 tetranucleotide and nine dinucleotide microsatellite data from the total Samburu, and a combination of all the 19 loci (because of small sample sizes) for the Kenyan individual and total subsamples

Table S3 Tests for deviation from the mutation–drift equilibrium on the Samburu total, its four cohorts, and a subsample of the Kenyan total and individual populations

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