

Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana

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Abstract

African forest elephants are difficult to observe in the dense vegetation, and previous studies have relied upon indirect methods to estimate population sizes. Using multilocus genotyping of noninvasively collected samples, we performed a genetic survey of the forest elephant population at Kakum National Park, Ghana. We estimated population size, sex ratio and genetic variability from our data, then combined this information with field observations to divide the population into age groups. Our population size estimate was very close to that obtained using dung counts, the most commonly used indirect method of estimating the population sizes of forest elephant populations. As their habitat is fragmented by expanding human populations, management will be increasingly important to the persistence of forest elephant populations. The data that can be obtained from non-invasively collected samples will help managers plan for the conservation of this keystone species.

Keywords: dung counts, genetic tagging, genetic censusing, microsatellites, molecular scatology, noninvasive sampling

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Introduction

Of the remaining 400 000–500 000 African elephants, one-quarter to one-third are forest elephants. In central Africa, forest elephants are currently referred to the species *Loxodonta cyclotis*, while those of west Africa belong to a newly recognized and yet to be formally named species (Eggert *et al.* 2002). Here we refer to this taxon as the western elephant. Because of the difficulties of studying them in the remote rain forests, elephants that live in forests are not as well known as those that live in savannas (Thurow 2002). Studies have shown, however, that forest elephants are seed dispersers for many plants (Alexandre 1977; Chapman *et al.* 1992; White *et al.* 1993) and are therefore important to the health and regeneration of the forest. They also create paths that are used by other animals and may be responsible for the creation and maintenance of the forest clearings where many species obtain essential mineral salts (Ruggiero & Fay 1994).

In the forest zones, expanding human populations compete with elephants for habitat. This is especially true in west Africa, where the future of forest elephant populations may soon depend entirely on protected areas (Barnes 1999). Unfortunately, forest elephants often feed at night in the fields surrounding forest reserves, resulting in hostility from local farmers and eroding support for their conservation. For these populations to persist alongside expanding human populations, management will become increasingly important.

Effective management requires data on population size, sex ratio, age structure and genetic variation. Since forest elephants are difficult to see in the dense vegetation, population sizes can only be estimated using indirect methods. The most commonly used method is the dung count, which relates elephant number to a count of dung-piles detected along transects, corrected for variables such as the deposition rate, decay rate and rainfall in the 2 months before the count (Barnes & Jensen 1987; Barnes *et al.* 1997). While some have questioned the accuracy of dung counts (Surendra Varman *et al.* 1995), Barnes (2001) found that when they can be compared directly to other methods, they produce population size estimates as

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precise as those obtained using any other method for a wide range of species. Nevertheless, they tell us nothing about the sex ratio, degree of relatedness between individuals and age structure of forest elephant populations. Further, they cannot reveal the level of genetic variability present, which is important in assessing the potential for the reduced survivorship and reproduction associated with inbred populations in some species (Saccheri *et al.* 1998; Woodruff 2001).

Several recent studies have used molecular markers to identify individuals from noninvasively collected samples for the purpose of estimating population size. Multilocus genotypes have been used as genetic tags, which have advantages over traditional tagging systems as animals cannot lose them, and there is no reason to believe that a noninvasively assigned tag will affect the ability to resample an animal. For dangerous or difficult-to-observe species such as bears (Kohn *et al.* 1995; Taberlet *et al.* 1997; Paetkau *et al.* 1998; Woods *et al.* 1999), marine mammals (Palsbøll *et al.* 1997; Reed *et al.* 1997), mountain lions (Ernest *et al.* 2000), wombats (Banks *et al.* 2002) and coyotes (Kohn *et al.* 1999), genetic surveys have provided valuable information for the management and monitoring of populations.

Noninvasive genotyping has several potential pitfalls, including false matches and false identifications. The problem of false matches, also known as the 'shadow effect' (Mills *et al.* 2000), occurs when individuals that have not been captured previously appear to be recaptures because their genotype is indistinguishable from that of a previously captured animal. False identifications can result from allelic dropout, where one of the two alleles of a heterozygous individual fails to amplify in a degraded or low-copy-number DNA sample, or from spurious alleles, which result when DNA from a source other than the target species is amplified (Taberlet *et al.* 1996; Gagneux *et al.* 1997; Palsbøll 1999; Morin *et al.* 2001). The resulting incorrect genotype cannot be matched to the correct genotype and will be identified as coming from a different individual. False identifications are more likely to occur when DNA is obtained from faecal samples, which may contain material from food sources as well as enteric bacteria (Bradley & Vigilant 2002).

For population size estimation, a further source of bias may exist when using noninvasive sampling. Rapid assessments of species in remote areas do not generally allow for an optimal unbiased study design. Animals that move a great deal may be sampled several times, while those that are more sedentary may not be detected at all. The heterogeneity of capture probabilities among animals may cause a negative bias in the estimation of population size.

To explore the possibilities of using noninvasive sampling to estimate population-level parameters, we conducted a genetic survey of the forest elephant population at Kakum National Park/ Assin Attandanso Game Reserve,

Ghana. The park is an isolated rain forest fragment and elephant immigration has probably not occurred for decades. Previous population estimates range from 100 to 150 individuals (Pajmans & Jack 1959; Dudley *et al.* 1992). Using multi-locus genotyping of DNA extracted from dung samples, we estimated the current population size, sex ratio, genetic variability and effective population size. We combined this information with field observations to generate an estimate of age structure. Finally, we teamed with the West African Elephant Biology and Management Project to compare our population estimate to an independent estimate generated using dung counts performed in both the wet and dry seasons.

Materials and methods

Sample collection

Kakum National Park and Assin Attandanso Game Production Reserve lie in the Upper Guinea forest zone in southern Ghana (Fig. 1A). Together they comprise 372 km² of moist evergreen forest. Although there is disagreement as to the exact date of their demarcation (Kpelle 1993; Hawthorne & Musah 1993), they have been 'reserved' since the 1930s. Logging, which began in the 1930s, was intensified in the 1950s and continued until 1989 when the Central Region Administration suspended all logging and transferred the reserves from the Forestry Department to the Game and Wildlife Department. In recent years, Conservation International's Ghana program, with financial support from the United States Agency for International Development, has worked closely with the Ghana Wildlife Department to develop the Kakum Conservation Area for eco-tourism. In this report, we refer to Kakum National Park and Assin Attandanso Game Production Reserve collectively as either 'Kakum' or 'the park'.

The Kakum elephants are believed to have been part of a much larger population that ranged throughout the forests of southern Ghana (Barnes 1993). There are no records of elephants to the south of the park area, but large numbers are believed to have lived in the forests to the north. These animals disappeared, however, following the construction of the railroad in the 1920s. Currently, Kakum is surrounded by agriculture and the elephant population is believed to be isolated as there are no elephants in any of the remaining small forest fragments nearby or in the Pra Suhien Forest Reserve, adjacent to Kakum (RFW Barnes pers. comm.).

To equalize sampling effort, we divided the park into 15 blocks of approximately 25 km² (Fig. 1B). A team of three to five people (LSE or RFW Barnes, one guide and one to three park game guards) spent 1 day in each, following elephant trails and collecting from as many fresh dung-piles as possible. To reduce our impact on the movement of

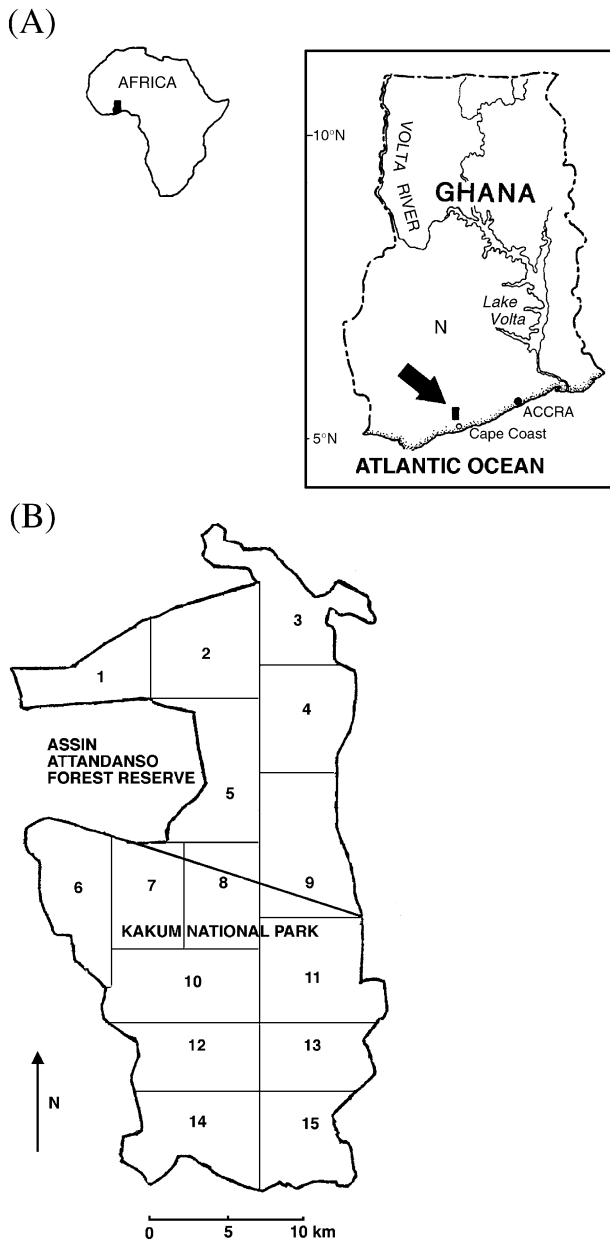


Fig. 1 (A) Location of Kakum National Park in Ghana, west Africa (from Dudley *et al.* 1992). (B) The park was divided into 15 blocks along latitude and longitude lines. Each block was sampled by following paths or elephant trails and using a GPS to lead us from the edge of the forest to the interior of the block, then returning using different paths. The boundary shown between Kakum National Park and Assin Attandanso is a cleared path.

animals in the park over the collection period, we randomized the order of collection of the blocks. Dung was aged using the criteria of Barnes & Jensen (1987), and only those of stages A–C1 were collected. This translates to dungpiles that are relatively fresh — our goal was to collect samples that were no older than 2 weeks. At Kakum the time from defecation to disintegration is affected by

rainfall (Barnes *et al.* 1997), but a study of the decay of dungpiles there showed that by 100 days all but a few had completely disintegrated. When dung had fallen as boli, the circumference of up to three boli per dungpile were measured and a portion was collected from both the outside mucus layer and from the inside of the bolus. We collected approximately 20 g in a 50-mL tube and added 20 mL of 70% ethanol as a preservative. In the field, samples were kept at ambient temperature.

Multilocus genotyping and sex determination

DNA extraction procedures were performed using a modified version of the protocol and reagents described in Boom *et al.* (1990). Extractions and amplifications were performed in separate UV-sterilized enclosures used only for low-copy-number samples. We centrifuged 1.5 mL of the dung in preservative for 15 min and discarded the supernatant. We then dried the sample for 30 min at 56 °C to remove any remaining ethanol, added lysis buffer L6 to a volume to 1.5 mL and incubated the sample overnight at 60 °C. We then centrifuged to pellet the debris and pipetted 750 µL of the supernatant into a new tube containing 250 µL fresh L6 buffer and 50 µL silica suspension. Samples were vortexed briefly and incubated at room temperature for 1 hour with shaking. After centrifuging for 3 min at maximum speed, we discarded the supernatant, washed the silica twice with 1 mL of wash buffer and once with 1 mL of 70% ethanol. The pellet was dried at 56 °C and DNA was eluted twice with 150 µL of sterile water. Each group of extractions was accompanied by control extraction blanks.

To ensure that ample elephant DNA was extracted, a 377-bp fragment of the 5' end of the mitochondrial control region was amplified using primers AFDL3 (5'-CTTCTTAAACTATTCCCTGCAAGC-3') and AFDL4 (5'-GTTGATGGTTTCTCGGAGGTAG-3'). Annealing temperature for this primer pair was 58 °C (Eggert *et al.* 2002). Each sample was extracted a minimum of twice and tested. Samples that failed to produce amplification products after the third extraction attempt were deemed unusable. A randomly selected set of 25 samples were then genotyped using six microsatellite loci developed for this project (Eggert *et al.* 2000) and five loci developed by Nyakaana & Arctander (1998). This initial survey showed that seven of the loci were polymorphic in the Kakum population.

Amplifications were performed in two steps. The first reaction was performed in a 5-µL volume containing 1.5 µL of DNA extract, 0.5 µL reaction buffer (Promega), 0.4 µM forward primer, 0.4 µM reverse primer, 0.5 mM dNTP mix, 1.5 mM MgCl₂ and 0.5 units *Taq* DNA polymerase (Promega). Using a Hybaid thermocycler, the profile consisted of a denaturation step at 94 °C for 3 min, followed by 40 cycles of 94 °C denaturation for 1 min, 1 min of primer annealing at a temperature 1 °C below the optimal annealing temperature

for the primer set and 1.5 min of primer extension at 72 °C. Immediately following the first reaction, samples (including the negative control sample) were reamplified by adding 5 µL of a labelled PCR mix containing 0.5 µL reaction buffer, 0.2 µM ³²P-γdATP labelled forward primer, 0.2 µM unlabelled forward primer, 0.4 µM unlabelled reverse primer, 0.5 µM dNTP mix, 1.5 mM MgCl₂ and 0.75 units *Taq* DNA polymerase. The profile was the same as above, except that the annealing temperature used was the optimal temperature for the locus. Control extraction blanks were included in the first set of amplifications for each locus and every set of amplification reactions included controls to which no DNA was added.

Alleles were separated in a 6% polyacrylamide gel, visualized by autoradiography, and scored by comparison with an M13 length standard and two control samples of African savanna elephants from the Frozen Zoo® of the Zoological Society of San Diego. For each locus, samples that were scored as heterozygotes were confirmed in a second reaction and samples that were scored as homozygotes were confirmed in at least two additional reactions. If there was an indication in any of the three results for putative homozygotes that there might be a second allele, we followed the multiple tubes approach of Taberlet *et al.* (1996) and analysed an additional four positive amplifications before scoring the genotype. Any set of reactions that showed amplification in the negative control was not scored.

To determine the sex of the sample we amplified 165 base pairs (bp) of a conserved portion of the SRY locus using primers SRYB and SRYB-3 (Pomp *et al.* 1995). As a positive amplification control for samples of both sexes, we multiplexed these primers with those for microsatellite locus LA2 (Eggert *et al.* 2000), which is monomorphic in this population at 205 bp. Samples that amplified the microsatellite locus three times without amplifying the SRY fragment were scored as females and those that amplified both the microsatellite and SRY twice were scored as males.

Population level analyses

Multilocus genotypes were entered into a file in Microsoft Excel 2000, where we used a Visual Basic for Applications (VBA) program to detect those that were unique. The first time a multilocus genotype was encountered it was scored as a 'capture', and each subsequent encounter was scored as a 'recapture' of that individual. Samples that would have been scored as a recapture of an individual within the same block were not included in the analysis. Sexing results were confirmed between captures and recaptures as a further check of their accuracy.

To quantify the power of our markers to distinguish between individuals, we computed the probability of obtaining identical multilocus genotypes for randomly chosen unrelated individuals, or the probability of identity

[P(I)]. This is based on allele frequencies at each locus, according to the following equation (Paetkau & Strobeck 1994):

$$PI \text{ (randomly chosen unrelated individuals)} = \sum p_i^4 + \sum (2 p_i p_j)^2$$

The multilocus P(I) is obtained by multiplying probabilities across all loci used. We produced a conservative estimate by ordering the loci from highest PI to lowest. However, using only the P(I) for randomly chosen unrelated individuals could underestimate the probability of finding identical genotypes in a population closed to immigration and emigration (Waits *et al.* 2001), as is believed to be the case at Kakum. We therefore also computed the P(I) for siblings, which represents the upper limit of the range of P(I)s in a population, using the following equation (Taberlet & Luikart 1999) to compute the value for each locus and multiplying across all seven loci, using the same conservative ordering of loci.

$$PI \text{ (siblings)} = 0.25 + \left(0.5 \sum p_i^2\right) + \left[0.5 \left(\sum p_i^2\right)^2\right] - \left(0.25 \sum p_i^4\right)$$

Mills *et al.* (2000) suggest that it will be difficult to distinguish between true heterogeneity of individual capture probabilities and heterogeneity detected because of the number of 'genetic shadows' of a particular individual. They point out that the traditional methods of reducing heterogeneity, increasing sample size and capture probability will not reduce the shadow effect. Reducing the P(I), however, will help to diminish the error associated with this phenomenon. Thus, we estimate that with seven highly polymorphic and independent loci we have minimized the chance that our results will be affected severely by genetic shadows.

Once the individuals in the data set were determined, we tested for Hardy-Weinberg equilibrium and genotypic disequilibrium using GENEPOP Version 3.2a (Raymond & Rousset 1995).

To estimate the population's age structure, we consulted Jachmann & Bell (1984), who determined the relationship between age and dung bolus circumference for 68 African (savanna) zoo elephants and for 38 wild African savanna elephants at Kasungu National Park, Malawi. They constructed age/dropping size curves for their data and used them to estimate the age structure of the Malawi elephants, then compared this method with an estimate derived from photogrammetry. Their results indicated that the method would likely under-represent young calves, but was otherwise a satisfactory method of estimating age structure. They found that there were differences in the size/age curve between zoo elephants and the Malawi elephants, indicating that the curve should be calibrated for different

populations. As body size and bolus circumferences have been shown to be directly related, we used this to estimate the relationship between age and dropping size for forest elephants. First, we compared the age and shoulder height data for forest elephants of known age (Morrison-Scott 1947) with that of the growth curves for savanna elephants (Lee & Moss 1995). At age one forest elephant calves were 90% as tall as savanna elephants, and by age 10 they were approximately 85% as tall. Between these ages, there was no significant difference between the height of males and females. The age at first reproduction is not known for forest elephants, but in healthy savanna elephant populations, individuals do not become reproductively active until after they are 10 years old. We thus estimated that forest elephants 10 years old or less would not be reproductively mature, and multiplied the bolus circumference of the age 10 Malawi elephants in the Jachmann and Bell study by 85% to arrive at a circumference of 32 cm for both male and female forest elephants at age 10. Because there is considerable room for error in assigning an absolute age to these animals, we discriminate only between a prereproductive class we call juveniles and those animals that are of reproductive age. Those with a bolus circumference at or below 32 cm were considered to be juveniles, while those above 32 cm were counted as adults.

To estimate the long-term effective population size (N_e), we inspected the distribution of alleles at all loci to determine whether it was more likely that they conformed to the expectations of the infinite alleles model (Kimura & Crow 1964) or the stepwise mutation model (Ohta & Kimura 1973). At all loci, we observed that either one or two alleles were at high frequency and all others were distributed at low frequency around the mode(s). We interpreted this as evidence that the appropriate model for estimating the long-term genetic effective size for this population was that of Ohta & Kimura (1973), based on the stepwise mutation model:

$$H_E = 1 - \left(\frac{1}{\sqrt{1 + 8N_e\mu}} \right)$$

where H_E is the frequency of heterozygotes expected under Hardy-Weinberg equilibrium, N_e is the genetic effective population size and μ is the mutation rate for the loci used in the analysis. All loci used were (CA)_n repeats, for which the mutation rate has been estimated at from 1.0×10^{-3} – 2.0×10^{-4} (Weber & Wong 1993; Amos *et al.* 1996).

Estimation of population size — synthetic data

Two general methods might be used to extrapolate from the number of unique genotypes to the estimated number of elephants at Kakum National Park. One involves an accumulation curve, in which a number of samples are

analysed and the curve is determined by the accumulation of unique genotypes. The asymptote of this curve is the estimated population size. The other method is mark-recapture analysis. To determine which method would give us the most accurate population size estimate under ideal circumstances, we compared two commonly used accumulation curve techniques with mark-recapture analysis, using synthetic data sets.

Our simulated populations contained 75, 150 or 300 individuals. We assumed that populations were closed (i.e. no immigration, emigration, birth or death over the time period of the study), that the area over which the population size was to be estimated had been sampled as completely as possible, and that all individuals had an equal probability of capture. Using VBA programs in Excel 2000 we selected randomly, with replacement, 100 sets of 125 and 250 samples from each population.

We used two common accumulation curve techniques, one of which assumes a hyperbolic form and the other an exponential form. Our use of the hyperbolic function (analysis E_{hyp}) to estimate population size is similar to the method used by Kohn *et al.* (1999) to estimate the number of coyotes in their study area in the Santa Monica Mountains of California:

$$E(x) = \frac{ax}{(b + x)}$$

where x is the number of genotyped samples, $E(x)$ is the cumulative number of unique genotypes found in x genotyped samples, a is the asymptote of the function and thus the estimated total population size and b is non-linear slope of the function which declines as x becomes large.

The second accumulation curve method (analysis E_{exp}) has been used to estimate species richness in a defined region, a problem analogous to the estimation of census size for a population. This model was used to census tree species in Costa Rica (Holdridge *et al.* 1971), and to estimate the species richness of rare vascular plants in the central southern Appalachians region (Miller & Wiegert 1989). Following the parameterization of the E_{hyp} equation, we estimated census size using:

$$E(x) = a(1 - e^{(bx)})$$

In Microsoft Excel 2000, we performed an iterative least mean squares regression fit of these equations to the data to estimate the values of a and b . As the order of addition of the samples can affect the estimation of the shape of the resulting accumulation curve (Colwell & Coddington 1994), each data set was randomized 100 times and the value of a was estimated each time. The estimate of a for the data set was the average of all iterations. Following

the recommendation of Colwell & Coddington (1994), we estimated the variance of a by computing the standard deviation for the data sets analysed for each population and sample size. As suggested by White *et al.* (1982), we computed the coefficient of variation as a measure of precision across different population and sample sizes. We also computed the percentage of bias for each population and sample size.

For mark–recapture analysis, we used the computer program CAPTURE (Otis *et al.* 1978), which includes models for closed populations where capture probabilities are constant (M_o), vary with time (M_t), vary with behavioural response to capture (M_b), vary by individual animal (M_h) or vary with a combination of these factors (M_{th} , M_{tb} , M_{tbh}). For real data, it is probable that heterogeneity exists from all three sources, making selection of an appropriate model neither simple nor straightforward (Otis *et al.* 1978). To select the best population size estimator, CAPTURE contains a procedure that analyses the data set using χ^2 tests for the goodness of fit of each model.

For each of the replicates, we compiled a capture and recapture history for each individual by dividing the dataset into 10 groups to simulate capture occasions. We then compiled a capture history for each individual, used CAPTURE's model selection procedure to evaluate each data set and recorded the results of the model suggested by the program. We computed the mean and standard deviation of the estimated census size for the 100 data sets analysed for each population and sample size, as well as the CV and percentage bias.

Estimating the size of the elephant population at Kakum

The size of the elephant population at Kakum was estimated using two methods. First, we used the accumulation curve method that performed best on the simulated data sets, E_{exp} . The data were randomized 1000 times and the value of a was estimated each time. The variance of a was estimated as the standard deviation among those results. For mark–recapture analysis, we considered our collections from each block as a capture occasion, and compiled a capture/recapture history for each of the 86 unique genotypes. Analysis was performed in CAPTURE using model M_h (Otis *et al.* 1978), as the model selection procedure detected significant heterogeneity in 'catchability' between capture occasions and between individuals.

Results

Multilocus genotyping, sex determination and population level analyses

Samples were collected from 205 dungpiles over the 15-day period. Because only very old dungpiles were found in

Table 1 Microsatellite loci used in this study. Loci LA3–6 are described in Eggert *et al.* (2000) and LafMS01–05 are from Nyakaana & Arctander (1998)

| Locus | No. of alleles | H_E | H_O |
|---------|----------------|-------|--------|
| LA3 | 3 | 0.521 | 0.527 |
| LA4 | 10 | 0.760 | 0.657 |
| LA5 | 7 | 0.575 | 0.377* |
| LA6 | 7 | 0.542 | 0.563 |
| LafMS01 | 6 | 0.501 | 0.518 |
| LafMS02 | 9 | 0.637 | 0.554 |
| LafMS05 | 3 | 0.328 | 0.382 |
| Mean | 6.4 | 0.552 | 0.511 |

*Significantly different from the expected heterozygosity value.

blocks 14 and 15, no samples were collected in those areas. DNA extraction was successful for 147 samples (72%), and for 124 (60%) we were able to amplify at least five of the seven loci and to determine that the sample did not have the same genotype as another collected in the same block. Of these 124, 86 were unique and 38 were scored as recaptures.

As we were unable to compare genotypes produced with dung with those detected from tissue samples, we estimated our genotyping error by examining the pattern of repeatability between amplifications. Of the first 738 single-locus genotypes, 116 (15.7%) did not match the genotype obtained using DNA from the second extraction. Within the first three amplifications of each locus, we were able to resolve all but three of these discrepancies as instances of allelic dropout. Using the multiple tubes approach, one of the remaining three was determined to be allelic dropout and two involved a single spurious allele. Direct sequencing of the three bands resolved for one individual confirmed that this 'allele' was not the target locus. Using these methods, we estimate that we reduced our cumulative probability of error to ≤ 0.0039 (0.157³), and that problems with allelic dropout and polymerase chain reaction (PCR) artefacts did not substantially bias our results.

We detected a total of 45 alleles at the seven loci, with an average of 6.4 alleles per locus (Table 1). At all but locus LA5, the observed and expected heterozygosity values were not significantly different from those predicted under Hardy–Weinberg equilibrium. The significant deviation at locus LA5 may signify the presence of one or more null alleles, which would make this locus less powerful in differentiating between individuals and could bias our estimate of heterozygosity and long-term effective size for this population. However, because this locus had seven amplifiable alleles it was useful in differentiating individuals and we believe any bias in estimation of population size would be to make the count more conservative. On

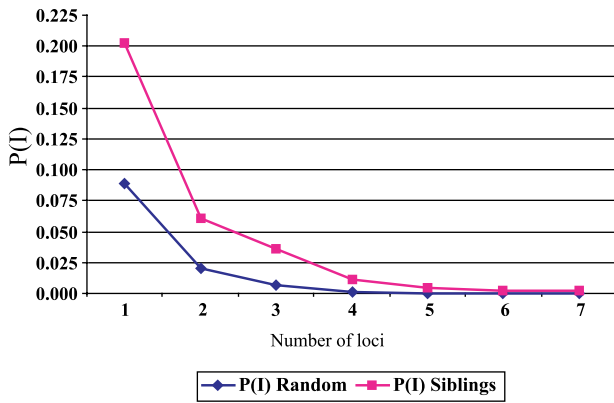


Fig. 2 The probability of identity [P(I)] for samples taken from randomly chosen unrelated individuals and from siblings as a function of the number of loci used in this study. To obtain a conservative estimate, loci were ordered from least to most informative.

the other hand, the bias introduced by null alleles in the estimation of heterozygosity and effective population size could be considerable, and we present the results of both analyses with and without this locus. The average observed heterozygosity value for the Kakum forest elephants was $\bar{H}_0 = 0.511$ when locus LA5 is included, and $\bar{H}_0 = 0.534$ without this locus. Of the 21 possible combinations of loci, there was no significant genotypic disequilibrium detected after a Bonferroni correction (Rice 1989).

With the seven markers used, we estimate that we would encounter randomly chosen unrelated individuals with the same genotype every 16 667 samples ($PI = 0.00006$), and

that we could encounter siblings with the same genotype once in 562 samples ($PI = 0.0018$, Fig. 2). These results indicate that our markers were adequate to differentiate between individuals at Kakum.

Using the sexing markers, we found that the 86 individuals comprised 35 males and 51 females, which is only marginally different from a 1:1 ratio ($\chi^2 = 2.9767$, $P = 0.0848$). When the age criterion was applied to the bolus size of the samples, we found that six of the males and 11 of the females were juveniles. The juvenile sex ratio was also not significantly different from 1:1 ($\chi^2 = 1.47$, $P = 0.2253$). We estimate that juveniles comprise 20% of the population.

The long-term effective population size depends on the mutation rate (μ) of the markers used, and is presented here as a range. Assuming μ falls in the range of 1×10^{-3} and 2.0×10^{-4} , the value of N_e is between 500 and 2489 when all loci are considered. When locus LA5 is deleted from the analysis, the range is 487–2434.

Population size estimation on simulated data sets

There were striking differences between the results of the two accumulation curve methods (Table 2, Fig. 3). Method E_{hyp} consistently overestimated population size and varied greatly among replicates. The analysis of more samples reduced the variance and percent bias, but even with the analysis of 250 samples for a population of 75 individuals, the population estimate was 132% of the simulated population size.

Method E_{exp} performed well in all cases, even when the sample size was small and the population relatively large.

| Method | Sample size | Population size | Mean | Range | SD | CV | % Bias |
|-----------|-------------|-----------------|------|------------|-------|------|--------|
| E_{hyp} | 125 | 75 | 117 | (88–155) | 11.8 | 10.1 | 56.0 |
| | | 150 | 261 | (184–369) | 37.7 | 14.4 | 74.0 |
| | | 300 | 648 | (377–1368) | 101.5 | 15.7 | 116.0 |
| | 250 | 75 | 99 | (91–106) | 2.8 | 2.8 | 32.0 |
| | | 150 | 229 | (200–266) | 13.8 | 6.0 | 52.6 |
| | | 300 | 515 | (419–648) | 46.5 | 9.0 | 71.6 |
| E_{exp} | 125 | 75 | 77 | (61–95) | 1.5 | 1.9 | 2.7 |
| | | 150 | 152 | (117–205) | 18.8 | 12.3 | 1.0 |
| | | 300 | 340 | (212–538) | 80.3 | 23.6 | 13.3 |
| | 250 | 75 | 75 | (70–79) | 1.8 | 2.4 | 0.0 |
| | | 150 | 150 | (135–172) | 7.8 | 5.2 | 0.0 |
| | | 300 | 300 | (250–357) | 23.6 | 7.9 | 0.0 |
| Capture | 125 | 75 | 76 | (62–137) | 10.7 | 14.1 | 1.3 |
| | | 150 | 151 | (116–249) | 25.8 | 17.1 | 0.7 |
| | | 300 | 319 | (203–540) | 72.7 | 22.8 | 6.3 |
| | 250 | 75 | 76 | (69–86) | 3.4 | 4.5 | 1.3 |
| | | 150 | 154 | (132–203) | 15.6 | 10.1 | 2.7 |
| | | 300 | 318 | (213–478) | 55.9 | 17.6 | 6.3 |

Table 2 Estimated population size using three methods on simulated data sets. Either 125 or 250 samples were taken from simulated populations of size 75, 150 or 300. One hundred replicates were analysed for each combination of sample and population size

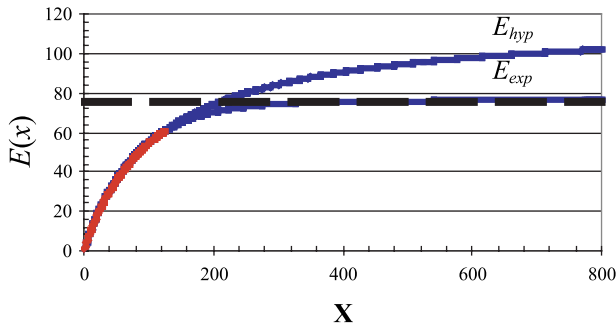


Fig. 3 An example of the results of population size estimation using asymptotic methods on simulated data, where X is the number of samples analysed and $E(x)$ is the population size estimate. The dashed line indicates the actual size of the sampled population (75) and the red segment indicates the portion of the curve determined by the 125 samples.

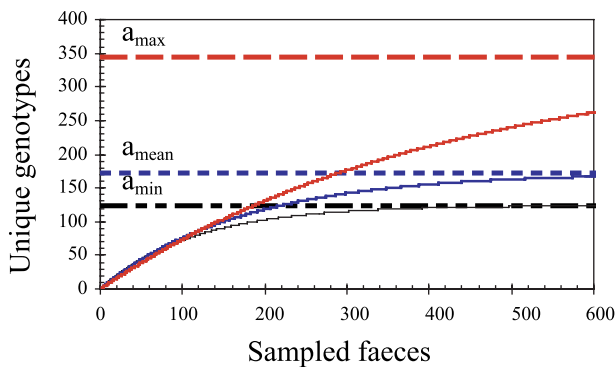


Fig. 4 Results of estimating population size for the Kakum elephants using model E_{exp} . Dashed lines indicate the asymptote values for the minimum (a_{min}), mean (a_{mean}), and maximum (a_{max}) estimates of population size.

Estimates of population size using this method were accurate and variance among replicates was much lower than was observed when the same data were analysed using method E_{hyp} . Increasing the sample size from 125 to 250 had little effect on the population size estimates in any case other than at the largest population size, but decreased the variance and percentage bias in all cases.

The model selection procedure in *CAPTURE* indicated that of the 600 data sets, 84% should be analysed using model M_{σ} , where capture probabilities are constant, and 15.3% should be analysed using model $M_{h'}$, which deals with capture probabilities that vary by individual animal. Three of the data sets were analysed using model M_{bh} and once the program selected model M_{tbh} . The results were accurate at all population sizes with little variance among replicates. When the sample size was increased to 250, 76% of the data sets were analysed with model M_{σ} , 22% with model $M_{h'}$, and 2% were either analysed with model M_{bh} or

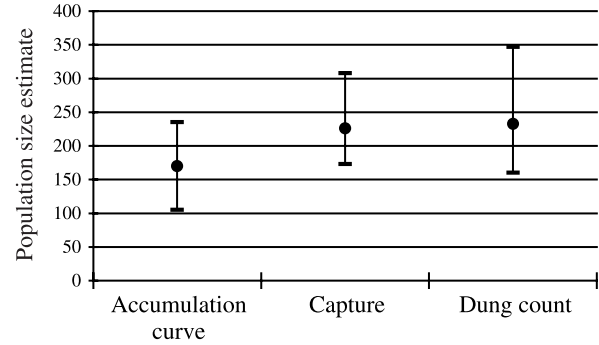


Fig. 5 Results of different methods of estimating population size for the Kakum National Park elephants.

were not analysed when model M_{tbh} (for which there is no estimator in the program) was selected. The increased sample size did not change the percentage bias for the estimates, but reduced the variability among replicates in all cases.

We compared estimates produced by all three methods using ANOVA, correcting for multiple tests using the sequential Bonferroni method (Rice 1989). Methods E_{exp} and mark–recapture consistently outperformed the E_{hyp} method ($P < 0.0001$). There were no significant differences between estimates produced using E_{exp} and mark–recapture.

Estimation of population size at Kakum

Using model E_{exp} we estimated that there were 170 ± 33.2 (SD) elephants at Kakum National Park (confidence interval 96–270, Fig. 4). We analysed the capture/recapture data for the 86 unique genotypes (Table 3) in *CAPTURE*. The model selection program identified heterogeneity in the data, with the capture probabilities (\hat{P}) varying from 0.00 to 0.20 per occasion. When the probability of capture varies by individual animal, the model best suited to estimating population size is the jackknife. We thus estimated population size using *CAPTURE*'s model M_h at 225 ± 33.99 (SE) with confidence limits of 173–308.

The Elephant Biology and Management Project provided their estimates from two extensive dung surveys done during 2000 (RFW Barnes pers. comm.). Their data were analysed using a model that relates the number of dung-piles in the forest to rainfall in the 2 months preceding the survey (Barnes *et al.* 1997). Their estimate during the dry season (February–March) was 239, with a confidence interval of 165–352, and during the wet season (October) it was 228, with a confidence interval of 158–337. These two values were merged (Norton-Griffiths 1978) to produce an estimate of 233 (160–347) elephants at Kakum National Park. The confidence limits were calculated using the method outlined in Barnes & Dunn (2002). All estimates are illustrated in Fig. 5.

Table 3 Capture histories for unique genotypes for the Kakum elephants. Recaptures of the genotyped samples are shown in bold type

| Day of Collection | Block no. | Male | | Female | |
|-------------------|-----------|-----------|-----------|-----------|-----------|
| | | Adult | Juvenile | Adult | Juvenile |
| 1 | 14 | None | None | None | None |
| 2 | 6 | 2 | 1 | 4 | |
| | | | | 3 | |
| | | | | 27 | |
| 3 | 9 | | 39 | 5 | |
| | | | | 6 | |
| | | | | 7 | |
| 4 | 12 | | | 8 | |
| | | | | 9 | |
| | | | | 10 | |
| | | | | 71 | |
| 5 | 15 | None | None | None | None |
| 6 | 1 | 11 | 25 | 5 | 12 |
| | | 13 | 39 | 14 | 15 |
| | | 19 | | 16 | |
| | | | | 20 | |
| | | | | 21 | |
| | | | | 35 | |
| 7 | 3 | 38 | | 36 | 37 |
| 8 | 2 | 13 | 25 | 5 | 18 |
| | | 19 | 29 | 8 | 28 |
| | | 22 | 39 | 10 | 34 |
| | | 23 | | 16 | 37 |
| | | 24 | | 17 | 48 |
| | | 30 | | 21 | 56 |
| | | 31 | | 26 | |
| | | 32 | | 27 | |
| | | 41 | | 33 | |
| | | 42 | | 35 | |
| | | 44 | | 40 | |
| | | 45 | | 43 | |
| | | 46 | | 47 | |
| | | 53 | | 49 | |
| | | | | 50 | |
| | | | | 51 | |
| | | | | 52 | |
| | | | | 54 | |
| | | | | 55 | |
| | | | | 76 | |
| 9 | 7 | 19 | | | |
| | | 57 | | | |
| | | 58 | | | |
| | | 59 | | | |
| 10 | 8 | 19 | | 60 | |
| | | 32 | | | |
| 11 | 11 | 19 | | 61 | |
| | | 62 | | | |
| | | 63 | | | |
| 12 | 10 | 57 | | | |
| | | 72 | | | |
| 13 | 13 | | | 64 | 34 |
| | | | | 65 | 67 |
| | | | | 66 | 68 |
| | | | | 69 | |
| | | | | 70 | |
| | | | | 71 | |

Table 3 *Continued*

| Day of Collection | Block no. | Male | | Female | |
|-------------------|-----------|-----------|-----------|-----------|-----------|
| | | Adult | Juvenile | Adult | Juvenile |
| 14 | 4 | 63 | | 20 | |
| | | 74 | | 73 | |
| 15 | 5 | 11 | 39 | 5 | 34 |
| | | 13 | | 17 | 37 |
| | | 25 | | 21 | 77 |
| | | 42 | | 26 | 81 |
| | | 62 | | 35 | |
| | | 75 | | 65 | |
| | | 78 | | 76 | |
| | | 83 | | 79 | |
| | | | | 80 | |
| | | | | 82 | |
| | | | | 84 | |
| | | | | 85 | |
| | | | | 86 | |

Discussion

Our experimental design is different than that of traditional studies that estimate population size. The samples were collected during a 'recce'-style survey, where the goal was to cover as much of the park as possible over the 15 collection days and to collect dung from as many individuals as possible. This style of survey is similar to those conducted by rapid assessment teams, whose goal it is to quickly assess the presence and absence of species in a region.

Although we sampled different areas of approximately 25 km² each day, each of these was small in comparison to the estimated range of an individual elephant. In Central Africa, Blake *et al.* (2001) found that the minimum convex polygon constructed from all known locations for a female forest elephant tracked using a global positioning system (GPS) collar over 45 days was 880 km². White (1994) suggested that forest elephants would travel 50 km to exploit seasonal fruits. The ephemeral nature of their preferred foods makes it highly likely that forest elephants move around a great deal within habitat patches. Because the size of Kakum National Park is only 372 km², well within the size of the potential range of a forest elephant, we have no reason to believe that we were collecting from different populations on different days.

There have been a number of studies and comparisons of asymptotic models that suggest which are most appropriate in a specific situation (e.g. Soberón & Llorente 1993; review by Colwell & Coddington 1994; Poulin 1998; Ulrich 1999). As it was difficult to reconcile these with the particulars of our work, and because we could find no a priori biological reason to choose one model over another, we chose to perform our own simple simulations, and to compare the results with those from mark-recapture analysis. Our results indicated that under ideal conditions model E_{exp}

performed best and provided results very similar to those from mark–recapture analysis. As it depends only on the probability of capture and does not deal with heterogeneity from any source, this model is actually a non-optimized (i.e. non maximum likelihood estimator) form of the null model (M_0) in CAPTURE, which explains the similarity of results.

The more difficult decision was whether the asymptotic method was comparable to mark–recapture analysis on our dataset. Asymptotic methods assume that the spatial distribution of individuals is random (Gotelli & Graves 1996). Although individuals are seldom randomly distributed in natural populations, randomizing the order of the samples 1000 times and calculating the mean value of estimates produced removes bias caused by the order of addition of samples.

These models also assume that sampling has been sufficient to represent the true collecting curve adequately (Gotelli & Graves 1996), which is more difficult to assess. Using rarefaction, we are extrapolating a curve that has not yet reached an asymptote. Figure 4 shows that there are considerable differences between the curves estimated by different randomizations of the data. Although the minimum value and the mean value are reasonable, some extrapolations produced very high estimates. As a result of this variability, we believe that our data may prevent us from representing adequately the true collecting curve.

In our sample, there was significant variance in ‘catchability’ between animals. Each genotype was detected an average of 1.4 times (range 1–5). This heterogeneity, which was identified by the model selection program in CAPTURE, could have been caused by our sampling procedure. Because elephants are known to use existing trails repeatedly, we followed paths and elephant trails throughout the park to ensure that we sampled as much as possible of the population. It is probable that this resulted in our sampling animals with large home ranges and those that moved around more often than more sedentary animals. We may also have missed smaller groups and lone males, while over-representing the larger groups, whose trails are more obvious to the human eye. Finally, we might expect heterogeneity in the capture rate of young calves (Jachmann & Bell 1984), whose defecation rate may be low until high-fibre browse becomes a significant part of their diet. Because of the heterogeneity of capture probabilities between individuals, we believe that the estimate obtained from the jackknife estimator in CAPTURE (model M_h) is likely to be more reliable than the one obtained from the asymptotic model.

Our genetic estimate is larger than the most recent estimate of 100–150 elephants at Kakum (Dudley *et al.* 1992). It is unclear whether this means that the population is growing or is a reflection of the fact that the Dudley *et al.* (1992) study, due to logistical and time constraints, surveyed only one part of the park and extrapolated over the entire area. As they themselves stated, the data they used were limited and their estimate was conservative.

Our estimates from both analysis methods are very close to the one obtained by the Elephant Biology and Management Project. Unlike previous models that have been used to relate dung density to population size, the model they used to analyse their results does not assume that the variables dungpile density, defecation rate and dung decay rate are normally distributed or that the forest is in a steady state (i.e. constant dungpile density from day to day). It relates the number of dungpiles to rainfall in the preceding 2 months (Barnes *et al.* 1997). Although dung counts have been compared with other methods in more open habitats (Surendra Varman *et al.* 1995), this is the first comparison with another method in the humid forest zone.

Although we are encouraged by the similarity between our population size estimate and the one obtained using dung counts, we caution that neither may be correct. Both methods require assumptions that may result in biased estimates. Our sampling design is similar to that used by rapid assessment teams. In general, rapid assessments of an endangered population in a remote area will not allow for an unbiased study design, and estimates produced are likely to be negatively biased. However, conservative estimates of population size may be better than overestimates, especially if managers are faced with potentially damaging decisions, such as whether or not they should reduce the size of a population through culling. Clearly, more studies are needed to assess the powers and pitfalls of noninvasive genotyping in estimating population size and demographic parameters.

We found that our juvenile age class included 20% of the individuals. Unfortunately, there are few studies of forest elephant populations that address age structure, and it is difficult to compare results across studies. Age and sex classes were determined using Moss’s (1988) criteria and Poole’s (1989) growth curves in a study at Odzala National Park, Congo (Querouil *et al.* 1999), while a study of the Lopé Reserve, Gabon, elephants does not describe the criteria used for classification (White *et al.* 1993). A study at Taï National Park, Ivory Coast (Merz 1986a) describes juveniles as having a shoulder height no more than 70% of that of an adult female, and at Bossematié Forest Reserve, Ivory Coast, elephants up to the age of two (as estimated by a forefoot circumference up to 50 cm) were classified as juveniles (Theuerkauf *et al.* 2000). In the first two studies, infants make up between 9.8% and 13.2% of the population, while juveniles and subadults make up an additional 21.2% to 23%. Juveniles at Taï constitute 30.5% of the population and the percentage of juveniles at Bossematié was found to be 15%. Our finding that 20% of the population are juveniles is thus reasonable, but not directly comparable with other populations. It is also possible that our use of dung to count animals underestimated the number of young calves due to their lower defecation rate. We can

Table 4 Forest elephant population densities (per km²) reported in West and Central Africa

| Location | Density estimate (elephants/area) | Reference |
|---|-----------------------------------|---------------------------------------|
| West Africa (Upper Guinea Forest Zone) | | |
| Bia National Park, Ghana | 0.33 | Barnes <i>et al.</i> (1998) |
| Sapo National Park, Liberia | 0.24 | Barnes & Dunn (2002) |
| Azagny National Park, Ivory Coast | 0.32 | Douglas-Hamilton <i>et al.</i> (1992) |
| Gola Forest Reserve E., Sierra Leone | 0.27 | Merz (1986b) |
| Bossematié Forest Reserve, Ivory Coast | 0.12–0.15 | Theruerkauf <i>et al.</i> (2000) |
| Gola Forest Reserve N., Sierra Leone | 0.10 | Merz (1986b) |
| Tai National Park, Ivory Coast | 0.01 | Hoppe-Dominik (1998) |
| Central Africa (Lower Guinea or Congo Forest Zone) | | |
| Lake Lobeke Forest Reserve, Cameroon | 0.56–2.1 | Ekobo (1995) |
| Lopé Faunal Reserve, Gabon | 0.90 | Barnes <i>et al.</i> (1998) |
| Banyang–Mbo Forest Reserve, Cameroon | 0.86 | Barnes <i>et al.</i> (1998) |
| Odzala National Park, Congo | 0.70 | Fay & Agnagna (1991) |
| Dzanga–Sangha Special Reserve and Dzanga–Ndoki Natl. Park, Central African Republic | 0.63 | Barnes <i>et al.</i> (1998) |
| Boumba Bek Forest Reserve, Cameroon | 0.50 | Barnes <i>et al.</i> (1998) |
| Korup National Park, Cameroon | 0.34 | Barnes <i>et al.</i> (1998) |
| Dja Faunal Reserve, Cameroon | 0.29 | Barnes <i>et al.</i> (1998) |

conclude, however, that this level of reproduction makes it likely that the elephant population at Kakum will be self-sustaining over the short term.

We estimate the average density of elephants at Kakum to be 0.61/km². This crude estimate is based only on the estimated number of elephants divided by the size of the park, and is likely to differ greatly between areas in the park. Nevertheless, it allows us to compare Kakum with other parks in the forest zones of west and central Africa (Table 4). Hunting for ivory and conversion of rainforest to agriculture have caused west African elephant population numbers to drop steeply since the 1940s (Merz & Hoppe-Dominik 1991; Roth & Douglas-Hamilton 1991). At Kakum, however, Dudley *et al.* (1992) found that there has not been elephant poaching since the 1970s, and that only a few crop-raiding elephants have been killed by Game and Wildlife officers. It is likely that Kakum provided a refuge for elephants that were displaced from surrounding areas as they were logged. A combination of factors including the absence of hunting, the slow recovery of the forest after logging and the absorption of displaced animals may have resulted in the Kakum population attaining a higher density than that of other west African populations, one that is more similar to that of central African populations (Table 4).

The average expected heterozygosity value of 0.552 for the Kakum elephants is less than, but within 1 standard error of, the average value (0.64 ± 0.14 SE) found for African forest and savanna elephants by Comstock *et al.* (2002). Their study found higher heterozygosity (0.77

± 0.11 SE) and a larger number of alleles per locus in central African forest elephants (13.8) than in savanna elephants (9.3) from eastern, southern and north central Africa. It is possible that the heterozygosity and allelic diversity of the Kakum forest elephants have declined over the last 70–80 years as the result of genetic erosion in an increasingly isolated population. Before we make any conclusions, however, we note that this is the first genetic study of a west African elephant population and it will be important to compare our results to those from other west African populations using the same markers.

Our estimate of the long-term effective population size for Kakum, from 500 to 2489, supports the idea that this population was once part of a larger, more contiguous population of forest elephants in the Upper Guinea forest zone. Although there may have been thousands of elephants present, large variance in breeding success among males and assortment of the population into family groups would reduce the ratio of N_e to N .

This study provides the first estimate of abundance and demography in a forest elephant population using genetic data from DNA extracted from dung. Given the inherent difficulties in collecting blood or tissue samples from elusive animals, noninvasive sampling and genotyping techniques promise to allow studies that would previously have been prohibited. Although more work is needed in the area of sampling design, we believe that studies such as ours will be beneficial to managers who seek to understand forest elephant population dynamics as well as the factors that affect crop raiding.

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