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ABSTRACT

Sixty-two years since last observed alive, *Mormotomyia hirsuta* Austen, the "terrible hairy fly", was found inside and outside a large, cave-like cleft boulder at the summit of Ukasi Hill in eastern Kenya, the type locality of the species. Adults were observed climbing the walls of the boulder and walking on thick layers of bat guano, in which larvae and puparia were also discovered. Large numbers of *M. hirsuta* were observed on and at the base of the northern side of the boulder, which at the time of capture experienced continuous shade during daylight hours. Only three individuals were observed at the southern opening, exposed to direct sunlight and hot, dry conditions. A collection of vertebrate bones and skulls from layers of guano both inside and outside the cleft revealed several vertebrate associates, including two species of Chiroptera, *Chaerephon* cf. *bivittatus* (Heuglin) and *Tadarida aegyptiaca* (E. Geoffroy), which are probably the two major guano-producing species responsible for the larval breeding medium. Male-biased sexual size dimorphism was pronounced in adult *M. hirsuta*, with seven body-part measurements, including legs, larger by 33–61 % in males than females. Males demonstrated isometric growth while female growth was allometric. In contrast to males, female head and thorax lengths did not increase proportionally with leg length. Estimates of genetic diversity in the Ukasi population show higher than expected allelic diversity and indicate possible gene flow and frequent population bottlenecks. To promote the conservation of this endangered species, a joint effort has been initiated between the International Centre of Insect Physiology and Ecology, Nairobi and the National Museums of Kenya, Nairobi, to gazette the Ukasi hill area as a protected site.

KEY WORDS: Mormotomyiidae, *Mormotomyia hirsuta*, Kenya, biology, biospeleology, cavernicolous, conservation, frightful hairy fly, genetic diversity, female allometry, sexual size dimorphism.

INTRODUCTION

Austen (1936) described a strange, hairy and brachypterous fly from two male specimens collected at Ukasi (as Ukazzi) in 1933 by Major Harry Barron Sharpe, then District Commissioner of the large Garissa District of eastern Kenya. The fly was characterized by its presumed association with bat guano, its greatly reduced and dysfunctional strap-like wings, its reduced eyes and lack of ocelli, and its superficially spider-like habitus. Although described as "spider-like" by Austen (1936), it perhaps more closely resembles a solfugid, at least when living and ambulant. Until 1948, two specimens deposited in the Natural History Museum, London remained the only known examples of this extraordinary species. In December 1948, following heavy rainfall, the species was again collected, this time in large numbers, by Victor Gurner

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Logan van Someren (1896–1976) (van Emden 1950). This collection, which included numerous specimens of adult females, larvae and puparia, was made at the type locality (Ukasi), apparently from the same large, split boulder at which Sharpe collected the original series (van Someren 1994).

Van Emden's (1950) description of the adult female and the immature stages, and his views on the phylogenetic position of the family, were based on this material. In the 62 years since the species was last collected, numerous efforts to relocate it at the type locality have been unsuccessful.

Mormotomyia hirsuta remains a species of considerable interest. The adult fly is truly bizarre-looking, with a vernacular name that, although somewhat hyperbolic, provides a relatively accurate physical description (*Mormotomyia hirsuta* = the frightful [hairy] fly (Austen 1936: 426, footnote)). Later, Oldroyd (1964: 184) was apparently the first to use the name "hairy terrible fly", the precursor to the currently used popular name, the "terrible hairy fly". The only representative of the dipterous family Mormotomyiidae (confined to the Afrotropical Region), *M. hirsuta* is currently considered the rarest fly in the world (F.C. Thompson, pers. comm. 2010), and is known only from the type locality. In addition, although van Emden (1950) reported that larvae were gua-nobious, and that adults occurred on the rock face inside the cleft, no additional be-havioural details were known. Of particular interest is whether the relationship of *M. hirsuta* with Chiroptera extends beyond larval nutrition alone, to adult phoresy. It is also not known whether other populations exist in similar habitats, or whether Ukasi Hill represents the only (relict) site. Finally, in the light of the contradictory evidence presented by morphological character states of both the larval and adult stages, the fly's systematic position within the order Diptera remains uncertain. Not surprisingly, this has generated diverse opinions regarding the phylogenetic position of the family (Austen 1936; van Emden 1950; Griffiths 1972; Pont 1980; McAlpine 1989; Grimaldi & Engel 2005; McAlpine 2007). Advances in molecular phylogeny (Yeates *et al.* 2007), held promise that the phylogenetic position of Mormotomyiidae within the Diptera could be resolved; a promise, however, that awaited freshly-collected specimens.

Some years ago, following inquiries regarding the location of the site, contact was made with the noted Kenyan botanist Quentin Luke, who not only knew of the site but was also able to provide GPS coordinates. In July 2008, a short visit to Ukasi (the currently accepted spelling of the area name), was made by one of us (RSC) and colleagues from the National Museum of Natural History (Washington DC, USA) and Iziko South African Museum (Cape Town, RSA) in an attempt to relocate the type lo-cality. During this visit two large boulders transected with clefts were identified that fit the description of the type locality provided by van Emden (1950), based on van Someren's personal observations. Attempts to locate living examples of *M. hirsuta* were unsuccessful at that time, however, possibly as this visit coincided with Kenya's dry season.

Two years later, in November–December 2010, a further expedition was conducted to the site, coinciding with Kenya's short rainy season; the period during which van Someren found flies in abundance. Here, an account is given of this successful 2010 expedition and follow-up expeditions in February and April 2011. Preliminary data are presented on male-biased sexual size dimorphism and allometric growth of females. Additionally, a list of cleft-inhabiting vertebrate associates of *Mormotomyia* is

included. Given the truly unique nature of this species (i.e. a single known, potentially ephemeral population), we address questions relating to the level of genetic diversity, evidence of recent genetic bottlenecks, the estimation of effective population size, and the potential existence of additional populations, through the development and
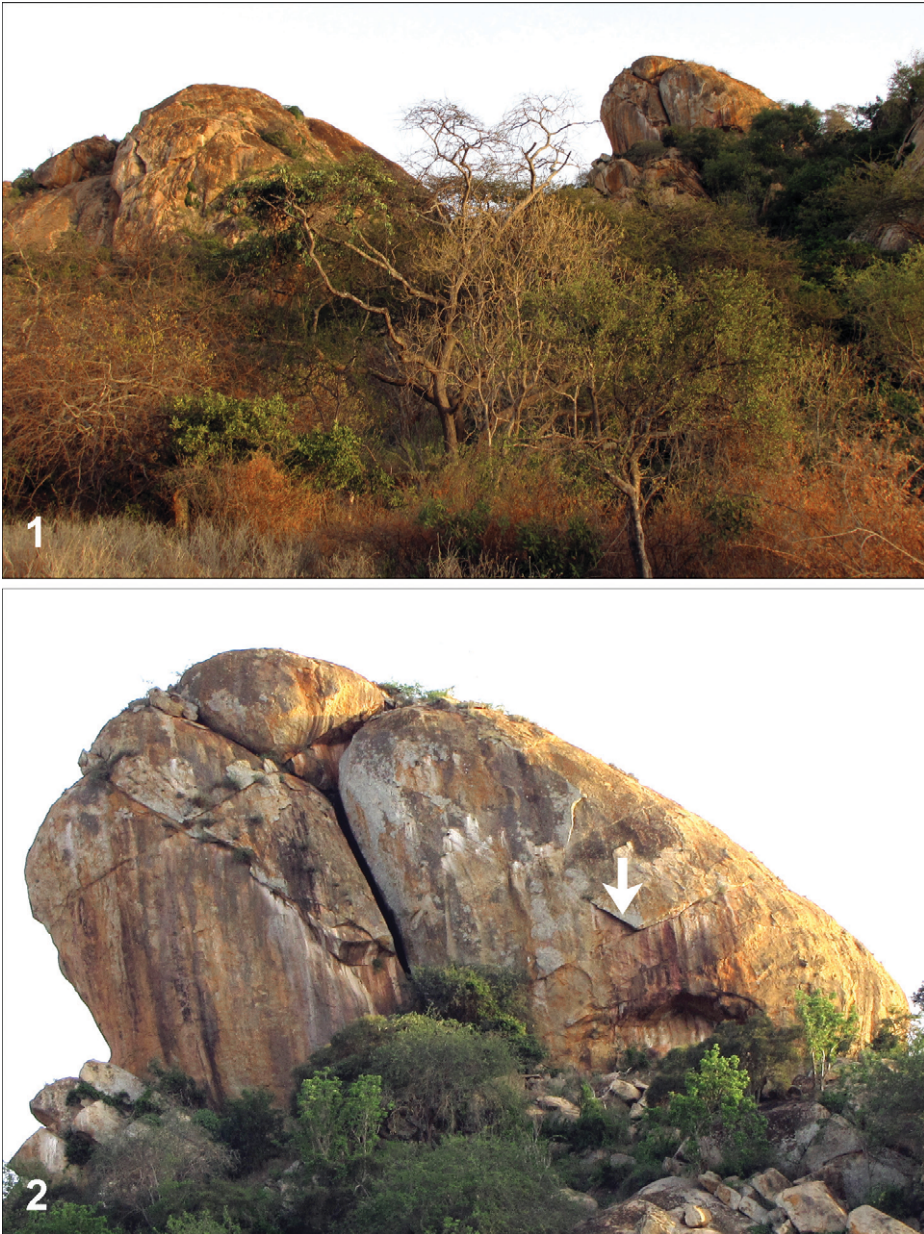
Figs 1, 2. Photographs of Ukasi Hill, Eastern Province, Kenya: (1) north face area with hillside *Acacia/Commiphora* vegetation; boulder with cleft at upper right of photograph; (2) detail of north face of cleft boulder; arrow indicates oblique crack in boulder. Not to scale. Photographs ©R.S. Copeland.

application of nuclear molecular markers, and through DNA sequencing of the mito-chondrial cytochrome oxidase I gene.

Details of larval and puparial morphology are presented elsewhere (Kirk-Spriggs *et al.* 2011), and molecular data bearing on the phylogenetic placement of Mormotomyi-idae within the Diptera will also be presented elsewhere (Wiegmann *et al.* in prep.).
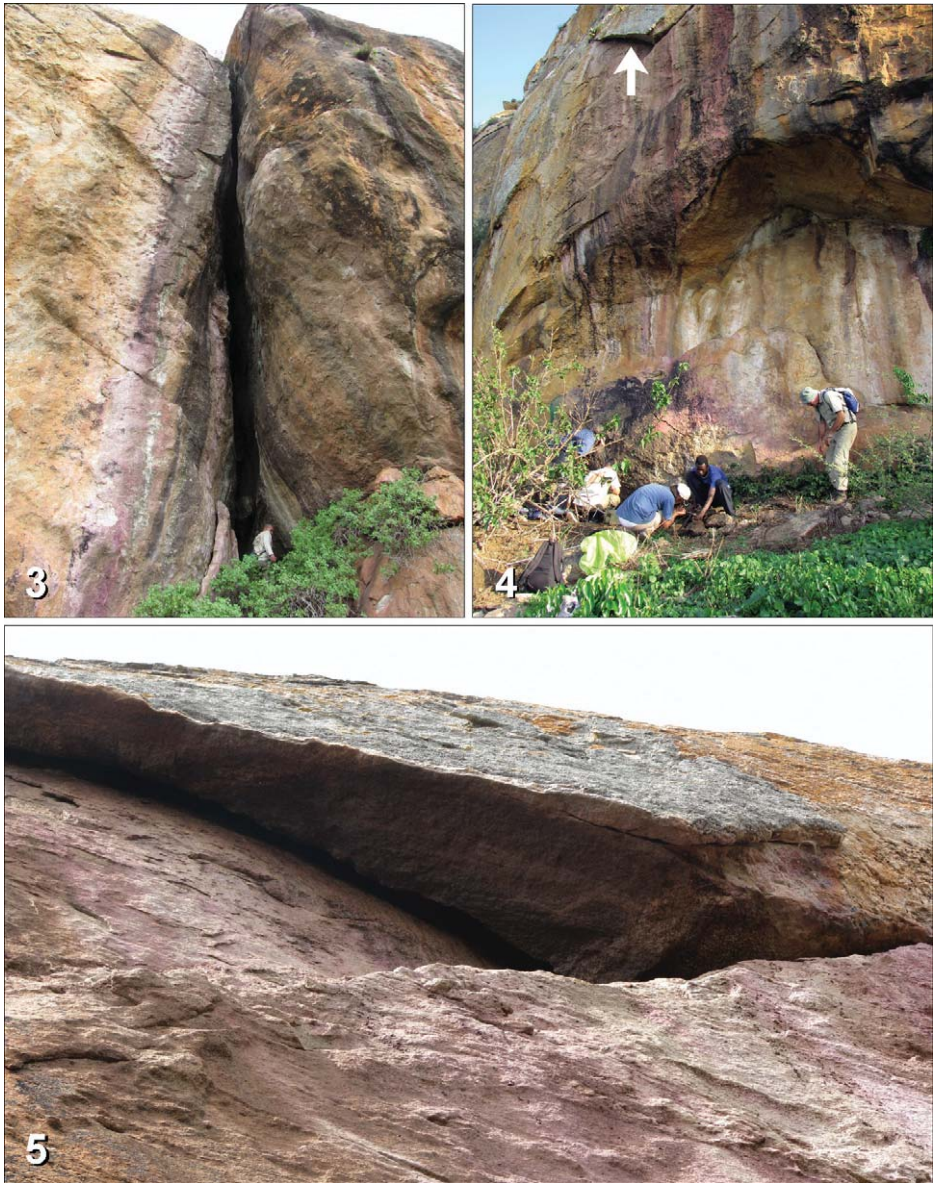
Figs 3–5. Photographs of Ukasi Hill: (3) cleft on southern face of boulder; (4) collecting *Mormotomyia* from bat guano at base of north face of boulder, arrow indicates oblique crack in boulder; (5) detail of oblique crack from immediately beneath. Not to scale. Figs 3 & 4 © R.S. Copeland, Fig. 5 © A.H. Kirk-Spriggs.

<div align="center">MATERIAL AND METHODS</div>

Site description and visits

Ukasi Hill lies in the immediate vicinity of Ukasi town, approximately midway on the Thika-Garissa road, and the hill and boulder where *M. hirsuta* was discovered are easily visible from the road. The boulder is positioned at the summit of the hill and is located at 0.81713°S:38.54225°E, at an elevation of 720 m (Fig. 1). The boulder is approx. 20–25 m in height, with a cleft running from top to bottom and more or less north-south, effectively splitting it in two (Figs 2, 3). Additionally, several small, oblique cracks with narrow openings are found only on the northern faces of the boulder (Figs 2, 4, 5). The area is generally hot and very dry, offering only marginal opportunities for agriculture. The vegetation is dry scrub with trees (Greenway 1973), with patches of *Acacia*/*Commiphora* woodland with *Aloe*, *Boscia*, *Maerua*, and *Sansevieria* spp. on the sides and base of the hill. Ukasi lies within the south-eastern branch of the Sahel that reaches as far south as northern Tanzania (Coe 1999).

On the first visit (30 November – 1 December 2011) the weather was hot and dry, but local inhabitants informed us that heavy rains had occurred in the area approximately two weeks previously. It was anticipated that there would be a need to simulate rainfall and the transportation of 120 litres of water to the top of the hill was arranged. This water was to be siphoned into the clefts to extract flies if necessary but, as conditions proved favourable, this was not required.

A second expedition was made to the site on 8–9 February 2010 to gather rock samples for geological characterization of the boulder and to explore within the large cleft, specifically to gather skulls, bones and feathers of vertebrate associates. Guano deposits spilling from the cleft on the north and south sides of the boulder, and up to three meters within the southern opening of the cleft, were examined. Penetration of the northern opening of the cleft was possible to about two-thirds of the length of the entire cleft (see below), over which distance guano was examined for evidence of associates.

A third expedition was conducted on 21 April 2011, when the long rains would normally have begun. Although light rain had fallen in the Ukasi region a few days earlier, the area was hot and dry when visited. Conditions within the large cleft were examined on the northern and southern sides. Evidence of the presence of *Mormotomyia* and of fresh bat activity was sought within the large cleft and on the ground outside the boulder, encompassing its entire perimeter.

Body measurements

Fifteen individuals of each sex were selected from specimens that had been collected live and held for 2–3 days before being killed by freezing. The largest and smallest specimen of each sex were chosen, while other individuals were selected randomly. Overall body length was not determined due to abdominal shrinkage. Two measurements (as indicated in Figs 6, 7) were taken using easily determined points of reference on the lateral side of the thorax and head. On the thoracic pleura a line was measured (A) from the dorsolateral edge of the anterior of the pronotum to the hindmost articulation of the hind coxa with the thorax. On the head a line was measured (B) stretching from the ventrolateral edge of the cheek to the vertex. A dorsal head
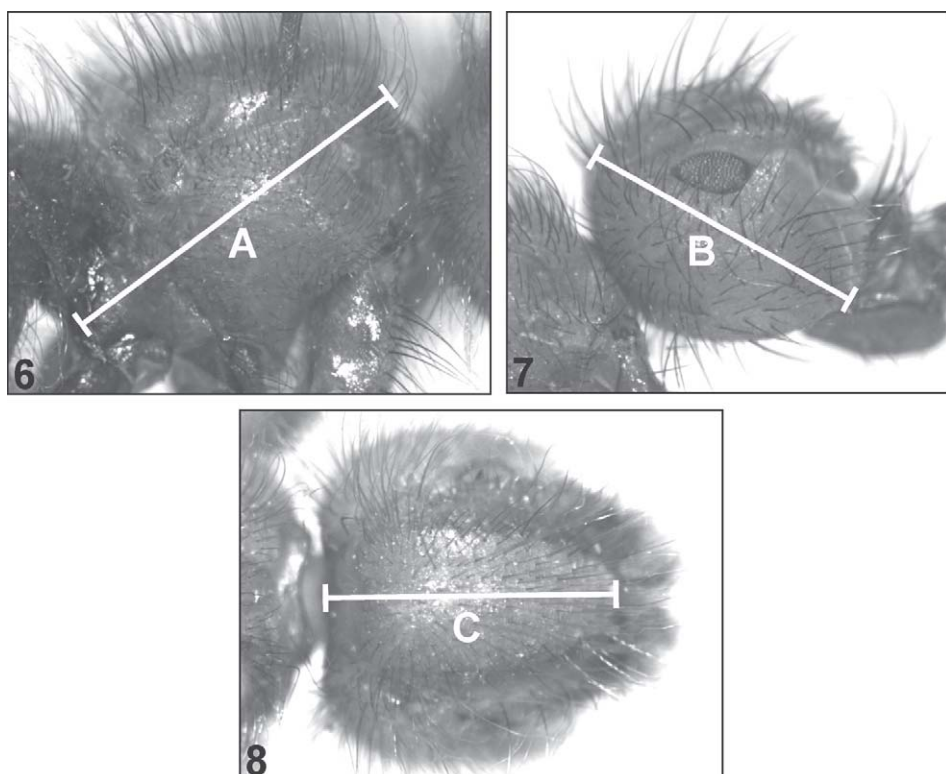
Figs 6–8. Adult measurements of *Mormotomyia hirsuta*: (6) lateral view of thorax; (7) lateral view of head; (8) dorsal view of head. Not to scale. Photographs ©R.S. Copeland.

measurement (C) was taken from the anterior edge of the frontal plate (easily visible) to the vertex (Fig. 8). Thoracic length (D) (not illustrated here), was measured along the dorsal midline from the anterior edge of the pronotum to the apex of the scutellum. The three right legs of each specimen were removed (when available) and were placed between two microscope slides to ensure that they lay flat. Femoral and tibial lengths were determined. Overall tarsal length was determined by summing the lengths of individually measured tarsomeres 1–5. Wings were not measured as these were too variably curled to confidently determine their lengths.

All measurements were made using ®LAS EZ software, version 1.5.0, on calibrated digital images captured on a ®Leica EZ4D binocular microscope. With the exception of female hind legs, lengths of body parts were distributed normally. Two-sample *t*-tests were undertaken to compare lengths of female and male body parts, except for hind legs, for which the non-parametric Mann-Whitney *U*-test was applied. To determine whether body part measurements of males and females were proportionally stable over the range of small to large flies, for each sex the metric data were fitted to a least squares regression model and the slopes of the lines of Log-Log plots examined (Futuyma 1986). In proportional (isometric) growth the slope approximates 1.0, while allometric growth is indicated when the slope deviates substantially from it; hypermetric when slope >1 and hypometeric when <1 (Shingleton *et al.* 2007).

Geology

Rock shards were chipped off the cleft boulder and submitted to the Geology Laboratory, University of Nairobi, for petrographic and mineralogical analysis. Standard mineralogical techniques were used, including microscopic analysis of thin-sections and atomic absorption spectrometry.

Genetic variation

Mitochondrial DNA sequencing and analysis

Flies were stored in 95% ethanol in an ultracold freezer before DNA extraction. Total nucleic acids were extracted from a single metathoracic leg from each individual specimen using the ®DNeasy DNA extraction kit (QIAGEN Inc., Valencia, CA, USA). Approx. 700 bp of the mitochondrial gene cytochrome c oxidase subunit 1 (CO1) was amplified using the primers LC01490f – GGTCAACAAATCATAAAGATATTGG and HC02198r – TAAACTTCAGGGTGACCAAAAAATCA (Folmer *et al.* 1994). Amplification conditions were as follows: initial denaturation at 95 °C for 60s; 35 amplification cycles (40s at 94 °C, 60s at 50 °C, 60s at 72 °C (following a ramp of 1 °C/second); 240s at 72 °C to complete the final cycle of amplification. PCR products were extracted from agarose gels and purified with the ®Qiaquick Gel Extraction kit (Qiagen, Santa Clara, CA, USA). ®Big Dye Sequencing kits (Applied Biosystems, Foster City, CA, USA) were used for sequencing reactions and sequencing was completed at the North Carolina State University Genome Sequencing Laboratory. Sequences were contiged and edited using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, USA).

Alignment was carried out manually using Se-Al 2.0 (Rambaut 2002). The final nucleotide alignment, translations, and phylogenetic data sets are available in Dryad (www.datadryad.org) and unique haplotype sequences are deposited in GENBANK (Accession Numbers JN398340–JN398361).

Haplotype diversity, nucleotide diversity per site, and the average number of nucleotide differences between unique haplotypes were calculated using the DnaSP v5 software (Librado & Rozas 2009). The programme TCS v1.21 (Clement *et al.* 2000), which follows the method outlined by Templeton *et al.* (1992), was used to determine the evolutionary relationships between mitochondrial sequences. A minimum spanning haplotype network was constructed with the connection limit between nodes calculated at 93%.

Microsatellite development

Following assessment of DNA quality and concentration, done with the ®BioSpec-Nano spectrophotometer (Shimadzu Scientific Instruments, Maryland, USA), pooled DNA from five specimens was subjected to shotgun sequencing using the ®Roche 454 Genome Sequencer FLX with the Titanium Sequencing kit XLR 70. Sequencing was performed at the North Carolina State University Genome Sequencing Laboratory. Sequencing was performed on a 1/4 GS FLX PTP.

A total of 441,628 reads were obtained with an average read length of 363 bp. Using MSATCOMMANDER version 0.8.2 (Faircloth 2008), all unassembled sequences were screened for di-, tri-, and tetranucleotide repeats using default settings within the program. Primers were designed using the PRIMER3 software (Rozen & Skaletsky 2000), implemented within the MSATCOMMANDER program, and tagged with a

19 bp M13 forward label (CACGACGTTGTAAAACGAC). Amplification products were chosen to be within a 100 to 450 bp range (including M13 tag), within an optimal annealing temperature of 59 °C (range 57–63 °C), an optimal GC content of ~50 %, low levels of self- or pair-complementarity, and a maximum stability (DG) of 8.0 (Faircloth 2008). Following the removal of duplicate sequences, a total of 14,379 sequences were found to contain tandem repeats within the desired criteria with sufficient flanking region for primer design: 2,749 di-, 10,766 tri-, and 864 tetra-nucleotide microsatellites with at least 6, 4, and 4 repeats, respectively. Of these, a total of 40 primer pairs were selected for testing (16 di-, 19 tri-, and 5 tetra-nucleotides).

Primer pairs were optimized using 5 individual *M. hirsuta* in order to minimize DNA depletion but maximize the likelihood of detecting polymorphism. Polymerase Chain Reactions (PCRs) were carried out in 12 μl total volumes, each containing $1\times$ PCR buffer, 1.75 mM $MgCL_2$ 100 mM dNTP's, 1 pmol primer, ~20 ng DNA template, 0.5U *Taq* DNA polymerase (Apex), and ddH_2O to 12 μl. The forward primer of each pair was end-labelled with an M13F-29/IRD700 or 800 IRDye tag (Li-Cor Inc). PCR cycling conditions were comprised of an initial denaturation stage of 3 minutes at 95 °C, followed by 28 cycles consisting of 30s denaturation at 95 °C, 30s at optimal annealing temperature of 59 °C, and 30s extension at 72 °C, carried out using a Multi-gene Gradient Thermal Cycler (Labnet International, Inc). Following PCR, 5 μl of stop solution (95 % formamide, 20 mm EDTA, bromophenol blue) was added to each reaction. Reactions were subsequently denatured at 95 °C for four minutes prior to loading onto a 25 cm 6 % polyacrylamide gel. Results were analyzed using the GENEPROFILER software (Scanalytics, Inc).

Unambigious PCR fragments within the expected size range exhibiting polymorphism were observed at 22 loci (Table 5). Subsequently, DNA was extracted from 24 *M. hirsuta* as described above, and screened at these loci.

Genetic data analysis

MICROCHECKER v2.2.3 software (Van Oosterhout *et al.* 2004) was used to assess the likelihood that null alleles, scoring error, and large allele dropout were evident at any locus screened. Basic summary population statistics (allelic diversity, expected and observed heterozygosity) were calculated using the Genetic Data Analysis (GDA) v1.1 software (Lewis & Zaykin 2002). Tests for departures from Hardy-Weinberg equilibrium (HWE) were calculated using the Genepop v3.4 software (Raymond & Rousset 1995). FSTAT v2.9.3.2 (Goudet 1995) was used to test for linkage disequilibrium and to calculate the inbreeding coefficient, F_{IS}, which measures departures from random mating within a population. The null expectation is zero and positive values can indicate two or more demes within the sampled organisms. It can also be an artefact caused by technical problems in detecting heterozygotes. It complements F_{ST}, departures from random mating among populations or demes. F_{IT}, the correlation of alleles in individuals sampled from a metapopulation, is $F_{IT}=(1-F_{IS})(1-F_{ST})$. Given the uncertainty that additional populations exist, F_{IS} and F_{IT} may be interchangeable, however in light of the results, for this manuscript F_{IS} will be used.

Population bottleneck analysis

Following a recent, severe reduction in a population's effective size (i.e., a genetic bottleneck), a characteristic genetic signature of an excess in heterozygosity may be

observed at selectively neutral genetic markers, such as microsatellite loci. This occurs because allelic richness declines faster than heterozygosity due to the loss of rare alleles that contribute little to the overall heterozygosity (Cornuet & Luikart 1996). To examine the possibility of the occurrence of a recent population genetic bottleneck, the distribution of allelic frequencies was analyzed following the Sign and Wilcoxon statistical tests, suggested by Luikart *et al.* (1998), which are implemented in the program BOTTLENECK v1.2.01 (Cornuet & Luikart 1996). As microsatellite loci are unlikely to strictly follow the stepwise mutation model, analysis was run assuming two alternative mutation models: the Infinite Allele Model (IAM), and the Two-Phase Model (TPM). The latter was run assuming 70 % single-step mutations.

Effective population size

The effective population size (N_e) is defined as the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. It is important to note that N_e is usually considerably smaller than the absolute population size (N), and as a result strongly influences a population's ability to respond to microevolutionary forces, such as genetic drift and bottlenecks. Given the inability to accurately determine the census size of the Ukasi fly population and in the absence of a temporal sample, estimates of N_e were derived following statistical procedures developed for application when only single samples are available. These estimates were generated using the recently-developed approximate Bayesian computation method, implemented in the software ONeSAMP v1.2 (Tallmon *et al.* 2008) (http://genomics.jun.alaska.edu). When running this program, we used priors of a minimum effective population size of 2, and a maximum effective population size of 2000. While previous methods employing one-sample estimators have proven imprecise or biased (Waples 1991; England *et al.* 2006), this method has yielded accurate results of effective population size in a vertebrate species following the screening of a comparable number of individuals and microsatellite loci (Tallmon *et al.* 2008).

RESULTS

Natural history and biology

Living adults of *Mormotomyia hirsuta* (Figs 9, 10) were located during the first expedition in 2010, but not during the subsequent two made in 2011. During the late afternoon of 30 November 2010 the base of the boulder on its northern side was examined. Movements and calls of bats could be heard from within one or more of the smaller oblique cracks. Large quantities of bat guano were discovered forming a thick layer in a small depression at the base of the boulder directly beneath the most prominent of these oblique cracks, this extending outwards for approximately 2 m, after which the flat ground fell away steeply. Adult *M. hirsuta* were immediately observed scurrying over the guano and along the face of the boulder beneath the crack (Fig. 4). Many of these flies were teneral and had clearly recently emerged from the guano deposit. The surface of the guano deposit was strewn with *ca* 80–100 dead adults, which presumably had recently emerged, but were unable to access the crack or the large cleft. Live adults were placed in plastic containers containing moist, ab-

sorbent paper or into 96 % ethanol for later molecular analysis. Some specimens were also preserved in 75 % ethanol. Larvae and puparia of *M. hirsuta* were collected from guano, and their treatment is described in Kirk-Spriggs *et al.* (2011).

Figs 9, 10. Live *Mormotomyia hirsuta*: (9) male; (10) female. Images captured in the laboratory on stones spotted with sugar solution. Not to scale. Photographs © R.S. Copeland.
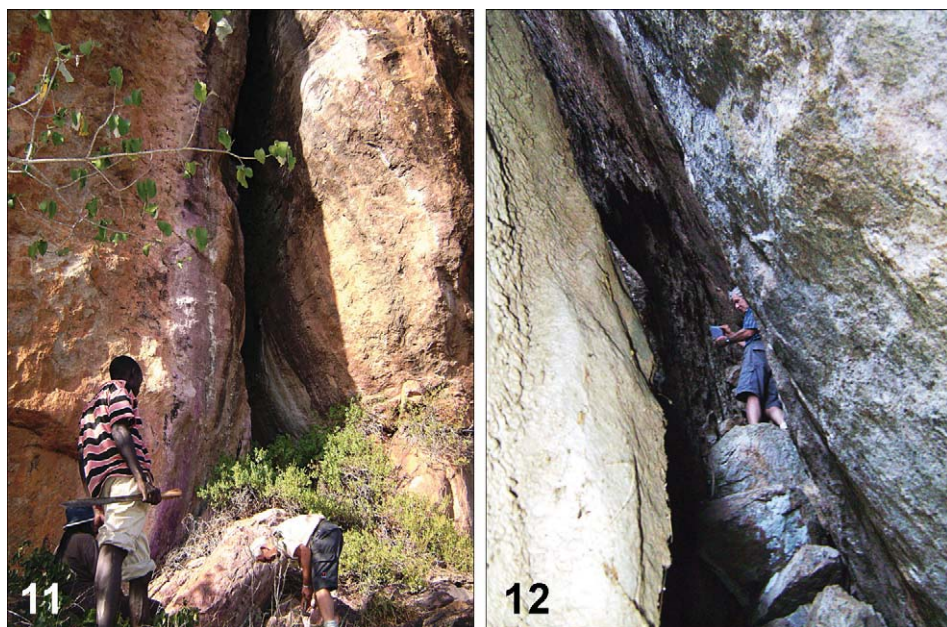
Figs 11, 12. Sampling sites and cleft openings: (11) opening of cleft on southern side of boulder from which three *Mormotomyia* adults were collected; (12) 'staircase'-like rocks extending up and into northern opening of cleft (four 'steps' are visible). Not to scale. Fig. 11 ©R.S. Copeland; Fig. 12 ©S. Muteti.

The following morning the expedition returned to the site at *ca* 10 am, the day remaining overcast until 11 am. A much larger aggregation of live flies was observed than the previous day. Although they were not systematically counted, it is estimated that approximately 600–800 live *M. hirsuta* adults were observed beneath the same oblique crack. Short digital movies were captured of adults walking rapidly along the rock surface.[1] The base and inside of the main cleft on the south-facing side of the boulder were also investigated. The opening on that side was considerably wider than that on the north side and allowed relatively easy human access. Thick accumulations of bat guano were also observed there, spilling from the cleft opening, but as this face was in direct sunlight it was very dry and only three live adult flies were observed, just within the cleft. Dry guano samples taken at this site later revealed empty puparia, indicating active breeding during favourably wet conditions. The opening of the main cleft on the north side could not be accessed as it was blocked with live and dead trees. However, copious amounts of guano that had been washed out of the main northern opening were searched for adult and immature stages of *Mormotomyia,* without success.

Characteristics of the boulder and the inside of the main cleft

During the second expedition rock shards were chipped off the boulder near the site of the northern and southern openings into the main cleft. The samples are parageneisses with nearly identical mineralogical content (Table 1), and are similar to the Quartz-Felspar-Biotite paragranulites that Wright (1964) recorded from the Ukasi area.

[1] http://www.youtube.com/watch?v=GZqFkAUyx5E; http://www.youtube.com/watch?v=dM8HuufOv8I

TABLE 1

The modal mineralogical composition of rock samples taken from the boulder at Ukasi Hill.

Mineral	Rock sample A (%)	Rock sample B (%)
Quartz	25	28
Plagioclase	24	22
Alkali feldspar	39	36
Biotite	7	6
Muscovite	2	2
Hornblende	1	1
Limonite	1	2
Apatite, Zircon, Sphene	1	2
Total	100	99

The cleft was easily entered on its southern side (Fig. 11), but after a few metres further progress was blocked by a large rock that had fallen from above. In order to enter the northern opening, dead wood blocking the entrance was removed. Inside the cleft, a series of similarly-sized rocks were wedged above the cleft floor against its two inner walls. These were arranged in such a way as to resemble a rising staircase and it was possible to proceed upwards into the cleft for about half its entire length (Fig. 12). This arrangement of "stairs" suggested that an entire rock slab had fallen when the boulder split open, the slab being broken into pieces when it smashed against the cleft walls that narrow below. About half way through the cleft the rock "staircase" continues downward toward the southern entrance. After proceeding about two-thirds the length through the cleft the rock "stairs" end abruptly and, without climbing ropes, a *ca* 15 m drop to the cleft bottom halts further progress. During the time inside the cleft (11h00–12h30), light penetration through the top of the cleft was considerable, making the use of artificial light unnecessary for exploration. Several bats were seen flying inside the cleft. They appeared to be light brown in colour, but this may have been an artefact of backlighting.

Vertebrate associates

To collect evidence of cleft-associated Chiroptera and other vertebrates, guano was closely examined at sites where this had washed out of the cleft, immediately within the entrance and on the relatively flat surfaces of the individual rocks making up the "staircase" within the cleft. All skulls, skeletal fragments and feathers documented there were collected inside the cleft, except for the hyrax remains and a single bat skull that were located only 2–3 metres outside the northern opening of the main cleft. Two species of bat, *Chaerephon* cf. *bivittatus* (Heuglin) and *Tadarida aegyptiaca* (E. Geoffroy) (Molossidae), were identified from complete skulls and teeth (Figs 13–18). A complete list of the vertebrate species collected is provided in Table 2. During two of the three expeditions to Ukasi, baboons were observed around the boulder. Although evidence of baboon activity is regularly observed in caves, there was no evidence of baboon activity within the cleft.
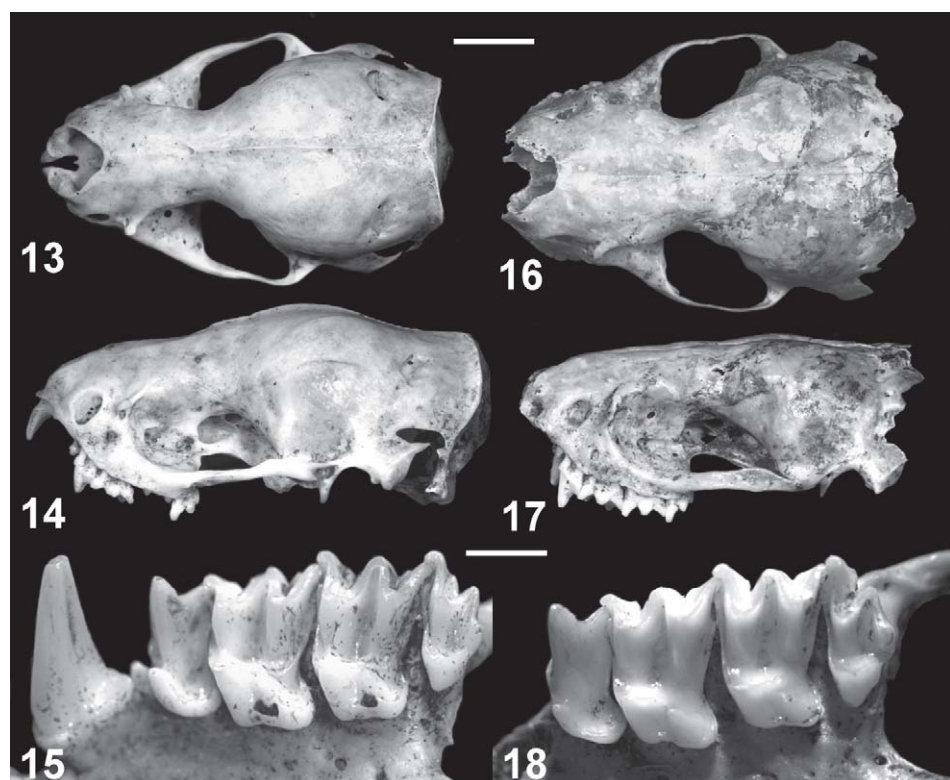
Figs 13–18. Skulls and teeth of Chiroptera collected in cleft at Ukasi Hill: (13) *Chaerephon* cf. *bivittatus*, dorsal view of skull; (14) same, lateral view of skull; (15) same, detail of dental structure; (16) *Tadarida aegyptiaca*, dorsal view of skull; (17) same, lateral view of skull; (18) same, detail of dental structure. Scale bars = 5 mm in Figs 13, 14, 16, 17; 2 mm in Figs 15, 18. ©R.S. Copeland.

The source of the guano that produced *Mormotomyia*

During the third visit to Ukasi there was no evidence of *Mormotomyia*. However, bat sounds were heard emanating from the same oblique crack beneath which we had collected the majority of the flies on our first visit, and very fresh guano covered the same area in a semicircular formation. Some guano fell from this crack while we were observing it, making it clear that bat activity within the crack, or simple gravity, is responsible for at least some of the accumulation on the ground beneath it. Fresh guano was also found just within the opening of the main cleft on its southern side where a few flies had been collected earlier. No other evidence of bat presence was seen, including within the northern length of the main cleft.

Sexual dimorphism in size of adult characters

For all body part measurements, *M. hirsuta* males were considerably and statistically larger than females, the percentage difference in length ranging from 33–61 % (Table 3). Although overall lengths were not routinely determined, due to abdominal shrinkage, overall body size (excluding legs) of the two largest males was measured. The lengths of these two specimens were 8.96 and 9.26 mm respectively.

TABLE 2

Vertebrate remains sampled in the cleft boulder on Ukasi Hill.

Class	Order	Family	Species[1]	Common name[1]	Identified material (n)
Mammalia	Chiroptera	Molossidae	*Chaerephon bivittatus* (Heuglin, 1861)	Spotted Free-tailed bat	skull (5)
			Tadarida aegyptiaca (E. Geoffroy, 1818)	Egyptian Free-tailed Bat	skull (1)
	Hyracoidea	Procaviidae	*Heterohyrax brucei* (Gray, 1868)	Small-toothed Rock Hyrax	skull (1)
	Macroscelidea	Macroscelididae	*Elephantulus* cf. *rufescens* (Peters, 1878)	Rufous or Spectacled Elephant shrew	tibia-fibula (1)
	Rodentia	Muridae	*Mastomys coucha* (Smith, 1834)	Southern Multimammate Mouse	partial skull from raptor pellet (1)
	Rodentia	Cricetidae	*Gerbilliscus* (*Taterona*) *robustus* (Cretzschmar, 1830)	Fringe-tailed Gerbil	skull (18)
	Rodentia	Bathyergidae	Unknown	Mole rat (Blesmole)	partial cranium (1)
Aves	Apodiformes	Apodidae	*Apus affinis* (J.E. Gray, 1830) or *Apus caffer* (Lichtenstein, 1823)	Little Swift or White-rumped Swift	feather (2) and skull (1)
	Passeriformes	Ploceidae	*Quelea quelea* (L., 1758)	Red-billed Quelea	skull (3)
		Sturnidae	*Onychognathus morio* (L., 1766)	Red-winged starling	feather (2)
Amphibia			Unknown species		leg bones (2)

[1] Scientific and common names from Encyclopedia of Life (http://www.eol.org; accessed 23 Feb. 2011).

TABLE 3

Sexual size dimorphism in *Mormotomyia.*

Body part[1]	Ratio ♂/♀	Mean length ± s.d. (mm) ♂	Mean length ± s.d. (mm) ♀	Statistical test	Value (t or U)	d.f.	p
A	1.33	2.38 ± 0.23	1.78 ± 0.14	t-test	8.35	28	<0.001
B	1.37	1.98 ± 0.23	1.44 ± 0.13		7.90	28	<0.001
C	1.45	1.42 ± 0.17	0.98 ± 0.07		9.28	28	<0.001
D	1.41	1.83 ± 0.26	1.29 ± 0.11		7.33	28	<0.001
Foreleg	1.61	11.37 ± 1.19	7.06 ± 0.66		11.57	26	<0.001
Midleg	1.47	11.1 ± 0.91	7.54 ± 0.77		11.03	26	<0.001
Hindleg	1.48	13.71 ± 1.33	9.27 ± 0.87	Wilcoxon rank sum test	195		<0.001

[1] See Material and Methods for explanation of body part measurements.

Allometric growth of female *Mormotomyia*

Correlation matrices of body and leg measurements of males and females are presented in Table 4. Both sexes exhibited positive, significant relationships among all non-appendage body parts measured (i.e., legs excluded). For males, lengths of all three legs were significantly correlated with each other and also with the four body part measurements. Additionally, Log-Log plots of male leg length and either head or thorax measurements had slopes near 1.0 (Figs 21, 22), indicating that growth of males was isometric; that is, larger males retained the same proportional size among body parts and leg lengths as smaller individuals. For females, however, the relationship between leg length and either head or thorax measurements was not statistically significant (Table 4, grey-shaded cells), and Log-Log plots produced flattened lines, with slopes much lower than 1.0, the expected value for isometric growth (Figs 19, 20). These data strongly point to female static allometry, and specifically to hypometric growth (*sensu* Shingleton *et al.* 2007); individuals with longer legs did not have larger heads and thoraces. Rather, growth appeared to be constrained in these two body parts.

Genetic variation within the Ukasi Mormotomyia *population*
Mitochondrial DNA

Overall, sequencing of the amplified COI region of 21 individuals yielded a 589 bp fragment suitable for haplotype network construction, resulting in six unique haplotypes. Haplotype diversity was estimated as 0.600 (standard deviation, 0.110), and nucleotide diversity per site as 0.0068 (standard deviation, 0.0024). Between haplotypes the average number of nucleotide differences was calculated as 4.052. The majority of the samples (13/21) exhibited a common haplotype (Haplotype A (Genbank accession JN398334)) (Fig. 23), or a closely related haplotype with one or two mutational steps (Haplotypes B (JN398335) (1/21) and C (JN398336) (4/21), respectively). From the transitional non-sampled haplotype between haplotypes A and C, arise three further haplotypes (D (JN398337) (1/21), E (JN398338) (1/21), F (JN398339) (1/21)), featuring between 12 and 14 mutational steps from the common Haplotype A (Fig. 23).

TABLE 4

Correlation matrices for male and female body part measurements.

Males

Measurement	A	B	C	D	Foreleg	Midleg
B	0.9228					
	p<0.001					
C	0.774	0.8093				
	p=0.0007	p=0.0003				
D	0.84	0.8561	0.8703			
	p=0.0001	p<0.0001	p<0.0001			
Foreleg	0.9186	0.8931	0.6918	0.7806		
	p<0.0001	p<0.0001	p=0.0043	p=0.0006		
Midleg	0.905	0.8835	0.6751	0.7904	0.9796	
	p<0.0001	p<0.0001	p=0.0057	p=0.0005	p<0.0001	
Hindleg	0.9265	0.9226	0.7264	0.8453	0.9529	0.9816
	p<0.0001	p<0.0001	p=0.0022	p=0.0001	p<0.0001	p<0.0001

Females

Measurement	A	B	C	D	Foreleg
B	0.907				
	p<0.0001				
C	0.7732	0.8573			
	p=0.0019	p=0.0002			
D	0.893	0.9396	0.8611		
	p<0.0001	p<0.0001	p=0.0002		
Foreleg	0.4635	0.2049	0.3024	0.3765	
	p=0.1107	p=0.5018	p=0.3152	p=0.2048	
Midleg	0.4523	0.2262	0.3589	0.3866	0.9775
	p=0.1207	p=0.4575	p=0.2284	p=0.1919	p<0.0001

Microsatellite DNA

Unambiguous genotypes were determined for 22 *Mormotomyia hirsuta* (Table 5). MICROCHECKER identified six loci as potentially exhibiting null alleles (Table 5). Due to the possibility that these loci may result in anomalous results, these were removed from population analyses. Following 2400 permutations, no significant evidence for linkage disequilibrium was detected. Given the unique nature of the population, allelic diversity and observed heterozygosity appeared moderate (allelic diversity range 3–9, average 6.31; observed heterozygosity range 0.208–0.875 average 0.638) (Table 6). Overall, the population was found to deviate significantly from Hardy-Weinberg equilibrium ($P=<0.001$), with one of 16 loci exhibiting significant deviations resulting from a deficit of heterozygotes (Table 6). Following 320 randomizations, the positive F_{IS} value of 0.109 was found to differ significantly from 0 ($P=0.003$), indicating inbreeding within the population. Bottleneck analysis revealed evidence for
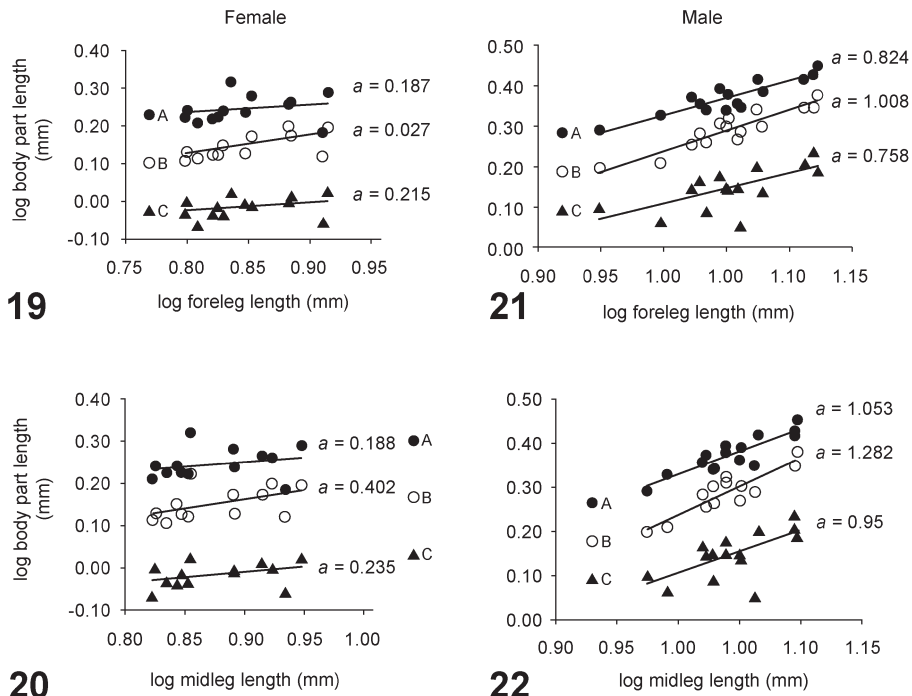
Figs 19–22. Isometry and allometry in *Mormotomyia hirsuta*: (19, 20) Log-Log plots of body and leg measurements of females; (21, 22) same for males. Abbreviations: A, B, and C refer to body part measurements illustrated in Figs 4–6. Slopes (a) of the lines log y = log b + a log x are indicated to the right of each line (see Material and Methods).

a recent severe genetic bottleneck under both the Sign (I.A.M.−P = 0.014) and Wilcoxon (I.A.M.−P = 0.005) tests. Estimates of effective population size calculated by the approximate Bayesian method yielded a mean Ne of 34.98 individuals, with 95 % credible limits for the posterior distribution ranging from 26.47 to 67.21 individuals.
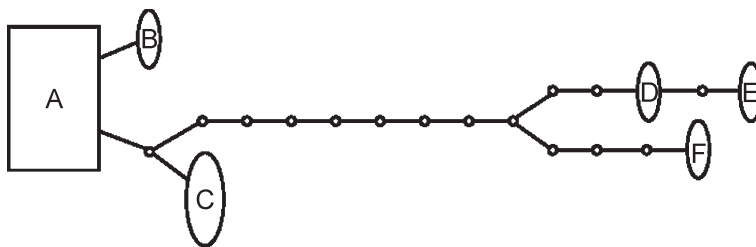

Fig. 23. Minimum spanning network calculated by TCS 1.21 using mitochondrial COI sequences. Haplotype with the highest frequency is displayed as a rectangle, while other, less frequency haplotypes are displayed as ovals. Circles represent haplotypes not sampled. Genbank accession numbers for haplotypes A through F are JN398334–JN398339, sequentially.

TABLE 5

Characteristics of 22 microsatellite DNA loci developed for *Mormotomyia hirsuta* and screened for a total of 24 specimens. *Locus with null allele as indicated by MICROCHECKER analysis (see Material and Methods).

Locus	Primer sequences	Repeat motif	Genbank Accession number
Mh4	F: CAATCTCCCGCGCATTGG	$(AAC)^{13}$	JN398340
	R: TGGTGGCTGACTCCTCTTG		
Mh6*	F: TCACTTGCATGTCCTTGCG	$(AAC)^{13}$	JN398341
	R: CATTGCACTTAGCCCTGCG		
Mh8	F: TGCCAAAGGAGTAAGGGCG	$(AAC)^{13}$	JN398342
	R: GCCAATTAGTCCGGCCAAC		
Mh9	F: ACTCCACAGACTGAGCGTC	$(AAC)^{14}$	JN398343
	R: TCCGAACCTTCGTATTCCC		
Mh11	F: GGTTGTCATCAAACCAACTGTC	$(AAC)^{16}$	JN398344
	R: ATCCGCCACGTTAGCCTC		
Mh13	F: AAATAGGTTGCTGTTTACCCTC	$(AAC)^{17}$	JN398345
	R: CGCGTGAAGAAAGATGCCC		
Mh14	F: AGACAGGCAAATGGGTACAG	$(AC)^{14}$	JN398346
	R: CACCATTCGTTTCCCTCAAATG		
Mh18	F: TGGAGGGTATGGTATATGGTAGC	$(AC)^{15}$	JN398347
	R: GCCTGACAAATCAGCTGCG		
Mh19	F: GACTTGAGTGTTGGAAGAGGC	$(AC)^{16}$	JN398348
	R: ATTCAGCTCATGTTGCGGG		
Mh20*	F: GAGGACCGCAAATTAGCCAC	$(AC)^{16}$	JN398349
	R: ACGATGTTTCGTGCACAGC		
Mh21	F: GTTGCTATTGCATGAGTTGGC	$(AC)^{16}$	JN398350
	R: GGCGGCTACCAAATCCTTATG		

DISCUSSION

The van Someren 1948 collection of Mormotomyia

The details of the 1948 collection of *M. hirsuta* probably differ slightly from the account offered by van Emden (1950). That paper refers only to V.G.L. van Someren using the opportunity of a trip to that part of Kenya to visit Ukasi Hill in the "… hope of discovering something about the biology of this interesting fly" (van Emden 1950: 121). However, the 1948 collection may owe more to chance than to purpose, and to two van Somerens, rather than one. In a letter dated 5 September 1994, G.R. Cunningham van Someren writes (van Someren 1994) that he accompanied his father on that expedition, the major purpose of which was to explore the region for butterflies in the area around Bura along the Tana River, south of Garissa. Heavy rain fell during

TABLE 5 *(continued)*

Locus	Primer sequences	Repeat motif	Genbank Accession number
Mh23	F: TGCCTAGCGGTAAGAAGGC	$(AC)^{13}$	JN398351
	R: TGGTGCATTTGGTCTTCCG		
Mh25*	F: GAGCCGCAACAACCTTTGG	$(AC)^{13}$	JN398352
	R: TCGTACTTTCACAATTGACTTCC		
Mh26*	F: ATTGTGGAATCCGCCCAAC	$(AC)^{14}$	JN398353
	R: GCTACAAATAGTTGCCCACTCG		
Mh28	F: AAGTAGGCGCTCACAGAGG	$(AC)^{14}$	JN398354
	R: TTGGCCGCCTTTCAATTTC		
Mh30*	F: CCACCATCAGCGTTTCAGG	$(AATG)^{8}$	JN398355
	R: GCCCGGTTGTAAGTAACGC		
Mh32	F: TGTTGGCTGTTGGTTCTTCAC	$(ACAT)^{10}$	JN398356
	R: TGTGCCTTACGAGCAGAATG		
Mh34	F: TGAGAAGCGCCAGCAAATG	$(AGGC)^{8}$	JN398357
	R: CCAACTTCTATTCTCCTGGAAGTC		
Mh36	F: CGTGTGCAGCTTAAACACTCC	$(AAC)^{11}$	JN398358
	R: GCAGGCAGTTTATGGTCTCG		
Mh37	F: CTCACTATCCTTCGTAGTCCC	$(AAC)^{11}$	JN398359
	R: GCTGCCAATGAGTGCTGAC		
Mh39*	F: GGCAATGGCAGTGATCTCG	$(AAC)^{11}$	JN398360
	R: TGAAGCCATCTTCTGATTTGGG		
Mh40	F: TGGCGCATTTGGTTATGGC	$(AAC)^{12}$	JN398361

the trip, and while returning to Nairobi the younger van Someren became unwell, the pair stopping overnight at Ukasi. In the morning they observed an "inselberg" on a nearby hill, and V.G.L. van Someren recognized the rock as that previously pointed out to him by H.B. Sharpe as the site of the latter's original discovery of the fly. The van Somerens explored the rock and the cave-like cleft together, finding the fly in great numbers on and near bat guano. It was also noted in the same correspondence that dual collection was not indicated on the specimen labels.

Natural history and biology
Conditions during the 2010 collection
    There was a marked difference in the numbers of flies between the north and south sides of the boulder during the 2010 expedition. Constant daytime shade helped maintain a favourable humidity gradient for larval development within the thick layer of guano that had accumulated outside the northern rock face. In contrast, guano immediately outside and within the larger southern opening was exposed to direct sunlight and very dry conditions, and this location appeared much less favourable for

TABLE 6

Summary statistics for *Mormotomyia hirsuta* population samples (n=22) screened for 16 microsatellite loci. A_n – number of alleles; H_E – expected heterozygosity; H_O – observed heterozygosity; F_{IS} – inbreeding coefficent; HWE – conformance to Hardy-Weinberg Expectations after Bonferroni correction; * – significant deviation resulting from an excess of heterozygotes; NS – non-significant P value.

Locus	A_n	H_E	H_O	F_{IS}	HWE
Mh4	7	0.753	0.640	0.153	NS
Mh8	9	0.831	0.708	0.150	NS
Mh9	7	0.789	0.875	-0.111	NS
Mh11	3	0.580	0.208	0.624	*
Mh13	9	0.834	0.826	0.009	NS
Mh14	6	0.702	0.640	0.090	NS
Mh18	9	0.651	0.583	0.106	NS
Mh19	8	0.765	0.727	0.051	NS
Mh21	4	0.650	0.480	0.268	NS
Mh23	6	0.790	0.609	0.229	NS
Mh28	6	0.575	0.591	-0.028	NS
Mh32	4	0.669	0.571	0.149	NS
Mh34	7	0.780	0.667	0.147	NS
Mh36	7	0.721	0.609	0.158	NS
Mh37	5	0.670	0.833	-0.250	NS
Mh40	4	0.631	0.636	-0.009	NS
Mean	6.31	0.712	0.638	0.109	$P=<0.001$

development at that time. It can be assumed that during the long rains (April–June) when the sun is on the northern side of the rock the situation would be reversed. Sharpe collected the original type material in May (Austen 1936). On a previous expedition to Ukasi in July 2008, *M. hirsuta* was not obtained. During that visit only the northern, sunny side of the boulder was investigated.

Development of *Mormotomyia* and its relation to source and condition of bat guano

It was curious that, during the first expedition, *Mormotomyia* was not found in the flow of guano that had been washed out of the northern opening of the main cleft. We did not closely examine this unproductive source but, in contrast to the area immediately below the oblique cleft, it did not contain a substantial portion of fresh guano. Presumably, colony movement within the main cleft changes from time to time, and current bat roosts are not located directly above the floor of the northern part of the cleft, as they are in the southern part.

Our observations in April 2011 indicate that the accumulation of guano outside the boulder is at least partly rainfall-independent. Indeed, it is difficult to see how rainfall could have penetrated the small oblique crack to wash out the guano on which most

of the fly specimens were collected. However, we did not witness a rainfall event at the site. Nevertheless, rainfall is clearly the force creating the flows of guano out of the northern and southern openings of the main cleft. Rainfall also provides the humid conditions necessary for larval development and is probably the major cue for the hatching of *Mormotomyia* eggs.

Vertebrate associates of *Mormotomyia*

Microscopic examination of guano pellets collected at the site revealed that these were exclusively composed of insect fragments. The two species of Chiroptera here regarded as the source of guano in the cleft at Ukasi, *T. aegyptiaca* and *C.* cf. *bivittatus*, are closely-related insectivorous species. Until recently, *Chaerephon* Dobson was regarded as a subgenus of *Tadarida* Rafinesque. *Tadarida aegyptiaca* is a widespread species, ranging throughout the drier parts of tropical Africa, northern Africa, the Middle East and southern Africa (Kingdon 1984*a*). In contrast, the distribution of *C.* cf. *bivittatus* is more restricted, the species occurring from Ethiopia to Zambia in eastern and central Africa (Kingdon 1984*a*).

Heterohyrax brucei (Gray) (Procaviidae) the Small-toothed Rock Hyrax, is known from the drier parts of Kenya. Hyrax droppings were concentrated on one of the rock "stairs" within the cleft, representing a communal latrine. Other such latrines occurred outside the cleft under rock overhangs. The single hyrax skull was located in a layer of bat guano approximately two metres outside the opening of the cleft. Both the Fringe-tailed Gerbil, *Gerbilliscus* (*Taterona*) *robustus* (Cretzschmar) (Muridae) and the Spectacled Elephant Shrew, *Elephantulus* cf. *rufescens* (Peters) (Macroscelididae) commonly occur in the drier parts of East Africa, with the former also extending across the Sahel to the Atlantic coast (Kingdon 1984*a*, *b*). *Elephantulus rufescens* commonly utilizes species of the dry-habitat succulent plants *Aloe* and *Sansevieria* for shelter and nesting (Kingdon 1984*a*), and both plants are common on Ukasi Hill. Neither *E. rufescens* nor *G. robustus* are particularly associated with caves. The presence of their skulls probably results from predator activity (if so, gerbils were the most common prey item in the cleft), or they may frequent caves in times of heat stress or to forage for invertebrates, possibly *Mormotomyia*.

Similarly, one can only speculate on the presence of the bathyergid bones and the *Quelea* skulls. The Taita Falcon, *Falco fasciinucha* Reichenow & Neumann (Falconidae), was known to nest on Ukasi Hill (van Someren 1994) and this small raptor preys on birds (Zimmerman *et al.* 1996), perhaps including the common *Q. quelea*. Interestingly, starlings (possibly the Red-wing Starling, *Onychognathus morio* (L.) (Sturnidae)) were also observed within the cleft during the 1948 visit by the van Somerens. The reflections of G.R.C. van Someren, as he recalled the events of December 1948, some 46 years later, are remarkably consistent with some of the identifications of bones and feathers we collected. In his 1994 correspondence he recalls his observations following entry into the cleft: "High up swallows, swifts and starlings flew in and out, bats of several species clung to the walls high up. A bonanza." (van Someren 1994). Of these noted taxa, only the presence of swallows in the cleft could not be verified.

Sexual size dimorphism

Sexual size dimorphism is common in insects, but females are usually the larger sex (Darwin 1871), size in females normally being positively correlated with fecundity.

Documented size dimorphism favouring males has, however, been described in many species. Increased male body size can result from sexual selection by females, with male size a proxy of fitness (Savalli & Fox 1998). Additionally, for species where males compete for females, larger males may have greater success in fighting off smaller males (Goldsmith 1987; Goldsmith & Alcock 1993), or in securing resources that make females more receptive to mating (Thornhill 1981). For some species, larger males which have already mated several times can continue to "switch off" further matings of previously uninseminated females, while smaller males having similar copulatory histories lose this ability, presumably because the latter's smaller volume of sperm has been exhausted (Cook 1992). A larger male may prevent access to a female by standing above her, behaviour Bonduriansky (2006) reported for the Australian neriid fly *Telostylinus angusticollis* (Enderlein), the males of which enclose females within the span of their legs during copulation and oviposition. Larger males have also been shown to have greater success than smaller conspecifics in locating females (Tammaru *et al.* 1996).

It is curious that van Emden (1950) made no mention of size dimorphism in *M. hirsuta*. He mentions (p. 121) the "… numerous adults of both sexes …" found by van Someren in 1948, on which material he [van Emden] made his observations, but did not indicate the sexes or number of specimens examined. It is possible he had too few to notice any obvious differences. Alternatively, specimens from this earlier collection may not have exhibited sexual size dimorphism. Environmental conditions at the site may have differed between 1948 and 2010, affecting growth. In any case, van Emden (1950) paid no attention to size in his paper, either absolute or relative.

It is impossible to know how the size of an individual male is determined by females or other males, but it is interesting to speculate on the function of the considerably longer, hair-like setae covering the greater part of the male (compare Figs 9 & 10). If the length of the setae increases proportionally to the size of an individual, then large males may appear larger still, lending them a further advantage if size is involved in sexual selection of males by females. Conversely, any increase in the perception of size that benefits mating may also be under selection pressure in the opposite direction by predators, which often differentially prefer larger insects (Whitman & Vincent 2008). At present, the functional significance of sexual size dimorphism in *M. hirsuta* is unknown. Its explanation depends on future *in situ* behavioural observations of live individuals.

Female allometry

In contrast to the males, females with longer legs do not have proportionally larger body parts (at least not the head and thorax). The hypothesis is suggested (subject to future testing on immobilized or freshly-killed females, prior to abdominal shrinkage), that an increase in female body size is limited to abdominal growth as an adaptation for increased fecundity (fitness). If true, an interesting implication is that, for females, growth of the head and thorax is uncoupled from that of the abdomen and legs. If this hypothesis is correct, then, presumably, in conditions favourable to larval development with sufficient quantities of moist guano, a critical size is reached for head and thorax, after which the genetic and physiological mechanisms controlling growth of the imaginal discs for these body parts are inhibited (Shingleton *et al.* 2007), while those

of the abdomen (and contents) continue to be active. Females with larger abdomens, but proportionately smaller heads and thoraces, would be produced.

Genetic variation within the Ukasi population

Despite the relatively small number of individuals available for molecular analysis, the genetic samples exhibited remarkable diversity at both the nuclear (allelic diversity and genetic heterozygosity) and mitochondrial (haplotype number and diversity) level. This is somewhat surprising given the nature of this population. Under an island model, it should be expected that a species would exhibit lower levels of genetic diversity compared to mainland populations of the same species (Frankham 1997). While the population investigated here effectively represents an "island", due to the species' apparent inability to undergo active dispersal, no comparable "mainland" population is available for direct genetic comparison. However, when compared to populations of other dispersal-limited invertebrates existing in relative, but not complete, isolation, diversity appears comparable (Crissman *et al.* 2010; Booth *et al.* 2011). These findings are contradictory to those predicted, given the population's apparent instability, as indicated by the evidence for a recent genetic bottleneck, its ephemeral nature, and likely re-establishment from minimal numbers of breeding individuals following population crashes, as indicated by the low estimate of effective population size. These data therefore suggest either an extremely stable population of randomly breeding individuals living within the cleft and cracks of the boulder, or genetic exchange from neighbouring, as yet undetected, populations. The former hypothesis is not fully supported due to the relatively low estimates of N_e that, as a result of the likelihood of overlapping generations, more accurately provides an estimate of the effective number of breeding individuals theoretically necessary to yield a population with comparable levels of genetic characteristics. As the mating system and reproductive output of this species become known, the estimates of N_e may in fact be sufficient to maintain the genetic diversity of an isolated population over time. The latter hypothesis, however, appears more plausible, given the findings outlined above from both the nuclear and mitochondrial analysis. At the mitochondrial level, we see moderate haplotype diversity, comparable to or greater than that observed in both island and mainland populations of other species (De La Rúa *et al.* 2001; Smith *et al.* 2006; McGaughran *et al.* 2008), and a significant number of mutational events among unique haplotypes. At the nuclear level we detect high levels of allelic diversity and a significant deviation from Hardy-Weinberg equilibrium. Thus, both mtDNA and nuclear DNA strongly suggest that gene flow may occur with other, as yet undetected, populations. Under this predication, missing intermediate mtDNA haplotypes may exist in as yet undetected populations, or, alternatively, may have been lost from the gene-pool following recent genetic bottlenecks and/or population extinctions. It is, therefore, tempting to speculate that gene flow may be attributed to the movement of flies, larvae, or eggs on cave-inhabiting vertebrates, to other roosts or nesting sites. Therefore, introduction of flies into the Ukasi population, resulting in gene flow, appears a more likely explanation of the observed variation.

Within the Ukasi population, genetic evidence also indicates the occurrence of inbreeding. This is not entirely unexpected following population re-establishment post-bottleneck, however it may also indicate the presence of multiple demes or breeding

units. The deviation from Hardy-Weinberg equilibrium resulting from an excess of homozygotes supports this theory. This apparent Wahlund effect is unlikely to result from the presence of null alleles, given that those loci with the genetic signature of possessing null alleles were removed prior to population genetic analysis. While this cannot be addressed further with the current data set, it is possible that this could arise through a number of population processes. These include niche/habitat partitioning, reproductive isolation between successive cohorts, or indeed, as suggested earlier, the occurrence of breeding individuals from other, as yet undetected, populations. Regardless of the population process responsible, the genetic estimate of effective population size suggests that the Ukasi population, whether founded from one or more groups, has resulted from a small number of individuals, supporting the positive F_{IS} estimate. Empirical studies utilizing the one-sample method for effective population size estimation have proved the precision of this method (Maudet *et al.* 2002; Tallmon *et al.* 2008), and values generated using the alternative linkage disequilibrium method (Waples 2006) have yielded comparable estimates (Aspi *et al.* 2009). We therefore do not doubt the current estimate generated, however we recognize that future studies incorporating additional samples, and temporal sampling, would prove valuable for addressing this question further.

At this time we can only speculate on possible modes of dispersal. *Mormotomyia* has none of the morphological adaptations for clinging onto bats' fur exhibited in other batfly families (Kirk-Spriggs *et al.* 2011). It is unlikely, therefore, that adult *Mormotomyia* are phoretic on bats or birds, although confirmation of this awaits examination of captured, living bats. One possibility is that *M. hirsuta* eggs are transported with guano on the feet of birds. Seeds trapped in mud are known to be dispersed by birds in this way (Darwin 1872; van der Pijl 1969) and *Mormotomyia* eggs are probably capable of remaining dormant for extended periods (Kirk-Spriggs *et al.* 2011). The guano observed at Ukasi was filled with developing larvae of *Mormotomyia* and other fly species, and it is unlikely that insectivorous birds would ignore such a rich source of food. Given the supposed isolation of the species (for millennia) there would have been multiple opportunities for events such as this to have occurred, even if other populations have subsequently become extinct.

As evidenced by the excess of heterozygosity observed at multiple loci following both the Sign and Wilcoxon tests, this population appears to have undergone a significant reduction in size, resulting in a genetic bottleneck. Given that genetic bottlenecks are only detectable for a short period following the decline, this reduction is likely to have occurred within the last 0.2 to 4.0 N_e generations, where N_e is the effective population size (Luikart & Cornuet 1998). If, in fact, this cave population is isolated, the long-term effect on this species' evolutionary potential could ultimately be detrimental (Frankham *et al.* 1999). The detection of a genetic bottleneck is consistent with the difficulty in detecting *Mormotomyia* during previous collecting expeditions undertaken throughout the 20th century.

The remarkable diversity observed at both the nuclear and mitochondrial level, in concert with the evidence for a recent genetic and thus demographic bottleneck, suggests that this population may in fact exist within a meta-population framework. Indeed, if past collection expeditions failed to collect specimens due to temporal extinction events within the cave, this would be explain their patchy appearance over

time. The large number of microsatellite DNA markers we developed will be extremely valuable for tracking and testing these hypotheses with flies from this population both temporally, throughout seasons and across years and, potentially, among other populations if, indeed, they are found in nearby localities in Kenya and other East African countries.

Conservation status of Ukasi Hill

As noted above, the area of Ukasi and its surroundings is hot and dry and, at best, provides only marginal prospects for agriculture. Wright (1964) stated that the mean annual precipitation on the western boundary of the Ndenyini area (where Ukasi is located) is "about fifteen inches" (*ca* 381 mm). Rainfall data for Sosoma, approximately 14.5 km southwest of Ukasi, was 330 mm between 1 January 1953 and 5 June 1954, and 445 mm between 19 April 1956 and 15 January 1957 (Wright 1964). At the time of Sharpe's discovery of *M. hirsuta* the area was sparsely inhabited, mostly by nomadic groups, although Ukasi itself had a small permanent human population prior to 1956 and, at that time, an impoundment already existed at the southern base of the hill (Wright 1964). Since then, as elsewhere, population has increased throughout the area, but damage to the hill does not appear to have been significant. Trees and shrubs on the hillside and in the flat area below do not appear to have been recently harvested for charcoal. Animal tracks are evident on the hill and it is likely that goats browse on vegetation, probably up to the entrance of the cleft. The fact that *M. hirsuta* was found in large numbers outside of the cleft, suggests that, currently, the Ukasi population is relatively robust. Nonetheless, the site is the only documented home of one of the world's rarest species and, even if other sites are identified, Ukasi is significant as the type habitat. Both Courtney *et al.* (2009) and Kirk-Spriggs & Stuckenberg (2009) specifically mention the imminent extinction threat to *M. hirsuta*. Accordingly, the site deserves special protection and the species should appear in the IUCN Red List of Threatened Species™.

Future research

The rediscovery of *M. hirsuta* opens up several promising avenues of research. This paper and that of Kirk-Spriggs *et al.* (2011) offer some insights into the biology and natural history of *M. hirsuta*. Additional research will test the reproducibility of the preliminary conclusions regarding male-biased sexual-size dimorphism and female allometry, and will determine the evolutionary advantages for maintaining these. Knowledge of the precise site of *M. hirsuta* development and the seasonal appearance of adults will allow us to focus on these and other questions. In what stage does *M. hirsuta* pass the very hot, dry seasons? Is *M. hirsuta* phoretic either on bats or, less likely, resident birds? Which bat species are resident in the cleft and responsible for the source of larval nutrition, and do these correspond to the species identified in this paper from skulls collected within the cleft?

Freshly-preserved material of this enigmatic species has now been made available to molecular geneticists/systematists to address two important questions: firstly, where does Mormotomyiidae belong in the Diptera tree of life, and secondly, is the Ukasi population entirely isolated, the only living relic of this strange family? The question of the phylogenetic placement of the Mormotomyiidae will be addressed elsewhere (Wiegmann *et al.* in prep.). In this paper, data on genetic variation revealed a surprising

amount of variability in the Ukasi population of flies, suggesting considerable gene flow. Do other cave systems in eastern Kenya and elsewhere harbour as yet undiscovered populations of this, or other *Mormotomyia* species? The numerous hills and inselberge spread about the landscape of eastern Kenya, of which Ukasi Hill is but one representative, are the visible remnants of ancient Precambrian rocks of the basement system that underlie the entire country (Cole 1950). Over geological time these other ancient rocks may have experienced the conditions that lead to the fracturing of rock, producing clefts and cracks similar to that found at Ukasi. Such habitats may conceal other populations of *M. hirsuta* or its relatives. Clearly, the opportunities for research on *Mormotomyia* are considerable, and the preliminary results presented here offer only a glimpse of the possibilities.

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