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Running Header: Social evolution in cryptomid mole-rats

A comparison of the population ecology of four *Cryptomys hottentotus* subspecies

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Selection pressures underpinning the evolution of mammalian sociality and body mass variation have spurred great interest for several decades. Because they inhabit a wide range of geographic localities and habitats, African mole-rat subspecies of *Cryptomys hottentotus* present a unique opportunity to further our understanding of intra- and inter-specific variation of sociality and body mass in mammalian species. We compared the demographics and body masses among four *C. hottentotus* subspecies: the Mahali mole-rat, *C. h. mahali*; highveld mole-rat, *C. h. pretoriae*; Natal mole-rat, *C. h. natalensis*; and common mole-rat, *C. h. hottentotus* within the context of their respective microclimates and evolutionary history. We propose that all ancestral *C. hottentotus* subspecies were arid-adapted and thus formed large colonies (as found today in the Mahali mole-rat). However, as the subspecies dispersed to occupy habitats of varying aridity and temperature across South Africa, selection for a particular colony size range in each subspecies arose to provide an adaptive fitness benefit to survive in its habitat. Consequently, the Mahali mole-rat—which remained in a warm and arid environment—retained the largest mean colony size, followed by the Natal mole-rat, which—even though inhabiting a hyper-mesic environment—selected for increased colony sizes to offset the energy requirement of thermoregulation (through huddling) in the cooler montane environments they prefer. The common mole-rat and the highveld mole-rat possessed the smallest mean colony sizes, likely the result of inhabiting a mesic and warm environment. At the same time, body mass variation in the *C. hottentotus* complex is likely linked to colony size variation, with subspecies having the largest colonies possessing the lowest individual body mass.

Key words: body mass, *Cryptomys*, mole-rat, population demographics, sexual dimorphism, thermoregulation

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Teaser Text: African mole-rats from the genus *Cryptomys* may hold the key to understanding proximate and ultimate factors behind mammalian social organization and body mass variation. Aridity and ambient temperature are considered strong selection pressures for colony living and body mass variation in various subspecies of *Cryptomys*.

Evolutionary theory posits that adaptations of an animal to its physical environment occur to increase the probability of survival of the individual and its offspring (Darwin 1859). Adaptations may be behavioural, morphological, physiological, and/or ecological in nature and may differ between and within species (Clutton-Brock 2021). In mammals, this has resulted in approximately 6400 extant species showing a wide range of adaptations. To better understand underlying proximate (ecological) and ultimate (evolutionary) factors behind these adaptations, research often focuses on particular orders, families, or species and particular adaptations. One such area of focus is variation in social organization—specifically colony structure or size (Ebensperger and Cofré 2001; Clutton-Brock 2021) and body mass (Blackburn and Gaston 1994, 1998; Hantak et al. 2021) in rodents—and in particular, African mole-rats in the Family Bathyergidae (Faulkes et al. 1997; Faulkes and Bennett 2013; Hart and Bennett 2023). African mole-rats are subterranean rodents endemic to sub-Saharan Africa occurring in underground burrows and demonstrate broad variability in social organization and morphologies, especially body mass (Bennett and Faulkes 2000). Social organization in African mole-rats ranges from strictly solitary to truly social (colony size range of 2–20 individuals in social species) to eusocial (colony size range of 2–300 individuals in eusocial species) species, while their adult body masses can range from an average of 35 to 780 grams (Bennett and Faulkes 2000). Even though extensive work has been conducted on the biology—including social organization and body mass variation—of

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African mole-rat species, some species have remained neglected, particularly within the genus *Cryptomys*. Variation between subspecies within this genus may further understanding of the proximate and ultimate factors behind social organization and body mass variation in African mole-rats and possibly other mammals.

The distribution, size, and digestibility of food resources (predominantly geophytes—underground plant storage organs) and predictability and abundance of rainfall have long been thought to play a crucial role in shaping sociality and body mass within the Bathyergidae (Jarvis et al. 1994; Faulkes and Bennett 2013). The importance of these ecological factors has given rise to the Aridity Food Distribution Hypothesis (AFDH; Jarvis et al. 1994). The AFDH posits that sociality in African mole-rats evolved from a solitary-living ancestor due to selection pressures for colonies by increasing survival through sharing the costs of foraging. The AFDH is centred around three core assumptions: 1) geophytes are usually larger, but occur at lower densities in more arid regions (Brett 1991; Jarvis & Bennett 1993; Spinks et al. 1999a; Lövy et al. 2012); 2) probability of finding food resources is lower in arid regions due to the clumped distribution of geophytes and blind foraging strategies of mole-rats (Lovegrove and Wissel 1988; Jarvis et al. 1998); and 3) energetic cost of burrowing and finding food is greatest in arid regions due to dry, hard soils (Vleck 1979, 1981; Lovegrove 1989). For species occupying arid regions, the potential for unsuccessful foraging has resulted in a tendency toward increased colony size and degree of sociality (Faulkes and Bennett 2013). According to the AFDH, food limitation reduces the body mass of individuals rather than number of animals in a colony (Jarvis 1985; Spinks et al. 2000a). This reduced body mass translates into substantial energetic savings for colonies in arid environments, potentially enhancing colony and individual survival (Spinks et al. 2000a).

The AFDH has been contested since some species in the genera *Fukomys* and *Cryptomys* (all colony-living) occur in mesic conditions and often sympatrically with solitary

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mole-rat species (Lövy et al. 2012; Šumbera 2019; Hart et al. 2022a). The AFDH predicts that mesic conditions should drive solitary lifestyles, yet some species persist as colony-forming mole-rat species (Bennett and Faulkes 2000). Consequently, other hypotheses have been developed to explain sociality in these mesic-dwelling social species and evolution of sociality in African mole-rats in general (Burda et al. 2000; Šumbera et al. 2007). More recently, Hart et al. (2022a) have expanded the AFDH by developing the Behavioural Osmoregulation Hypothesis (BOH). The BOH combines energetic (AFDH) and osmoregulatory benefits of colony living, essential factors to account for considering the added constraint that mole-rats do not drink freestanding water but obtain all of their water requirements from underground geophytes (Bennett and Jarvis 1995; Spinks et al. 1999a; Malherbe et al. 2003). As such, the BOH suggests that in species that form large colonies, water and food (energy) are not limiting resources as increased colony size allows for the efficient locating and retrieval of food and water (Hart et al. 2022a). Therefore, an increased colony size would be found in species or subspecies inhabiting arid regions to allow for sufficient food and, ultimately, water gathering (Hart et al. 2022a). Consequently, Hart et al. (2022a) hypothesized that ancestors of mesic-dwelling social mole-rat species may have been arid-dwelling social species that moved into a mesic environment, and since there is an energetic (including possibly thermoregulatory) and osmoregulatory benefit to remaining as colony-forming species, they retained a social lifestyle (Faulkes et al. 2004; Hart et al. 2022a; Visser et al. 2019a, 2019b). However, a comprehensive understanding of the possible evolutionary theory behind sociality and how this may link to body mass in African mole-rats is still lacking. Therefore, further studies evaluating the link between sociality (colony living), body mass, and habitat conditions in African mole-rats in species occurring in both mesic and arid habitats are critical. *Cryptomys hottentotus* is an excellent group of model organisms to investigate such links due to their wide geographical range and occupancy of

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varying climatic zones.

This study, therefore, aimed to record colony size and subsequent population density, colony biomass, age class distribution, adult sex ratio, litter size, and body masses of three subspecies of *C. hottentotus*: Natal mole-rat (*C. h. natalensis*); highveld mole-rat (*C. h. pretoriae*); and Mahali mole-rat (*C. h. mahali*) that live in vastly different environments (semi-arid to hyper-mesic), while also comparing results to previously published work on the common mole-rat (*C. h. hottentotus*) from both a hyper-mesic and arid environment (Spinks et al. 2000a, 2000b; Table 1). In addition, to comprehensively explore possible factors affecting body mass, we included reproductive status and sex as influencing factors. Lastly, as it is believed that abiotic climate factors (ambient temperature, soil temperature, and rainfall) have led to possible subspecies differences, we have included how seasonal (wet and dry season) variation may influence all measured biological variables in highveld, Mahali, and Natal mole-rats (see Supplementary Data SD1 for evidence on evolutionary history of the *C. hottentotus* complex).

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Materials and methods

Study sites and capture methods.

Highveld mole-rats were captured at the National Botanical Gardens, Pretoria, in Gauteng Province (Gardens, 25°44’ S, 28°16’ E); Mahali mole-rats were captured on communal land on the border of the North-West Province and Gauteng near Brits (Brits, 25°38’ S, 27°55’ E); and Natal mole-rats were captured at Glengarry Holiday Farm in KwaZulu-Natal Province (Glengarry, 29°19’ S, 29°42’ E). The National Botanical Gardens and Brits have similar soil types and precipitation patterns and are characterized as Marikana Thornveld (Mucina and Rutherford 2010; see results). Glengarry is characterized as Drakensberg Foothill Moist

Grassland (Mucina and Rutherford 2010). The propensity of mole-rats to occupy cultivated farms may bias body mass, colony persistence, and population ecology since farming affects soil moisture and mortality of mole-rats and other rodent species in sub-Saharan Africa (Wale et al. 2016; Ramahlo et al. 2022). Thus, we made a concerted effort at all study sites to focus on natural ecosystems to reduce anthropogenic effects. As such, each study site was chosen to represent native vegetation of the site, with limited to no supplemental watering or maintenance of vegetation or soil.

We captured mole-rats at each study site using Hickman traps (Hickman 1979) during four biannual excursions per subspecies between 2019 and 2021, with two trips to each site during the wet season (November – April) and two during the dry season (May – October). At capture, mole-rats were sexed, weighed, and implanted with a subdermal passive integrated transponder for identification (highveld and Mahali mole-rats, IdentiPet, Johannesburg, South Africa; Natal mole-rats, DorsetID, Aalten, The Netherlands). Animals were scanned at capture with a handheld transponder-reader for presence of a transponder and provided with one if newly captured. Each mole-rat colony was housed independently in a plastic box with wood shavings and fed with sweet potatoes *ad libitum*. A colony was deemed entirely captured out (a complete colony) if no trap activity (soil in traps, food eaten, or trap triggered) was observed within 48 hours after the last animal was captured. After 48 hours of inactivity, or seven total days in captivity, animals were released back into their home burrow. If we found signs of activity in traps after seven days, we stopped trapping and released animals to avoid undue stress, and these captures were considered incomplete if reproductive animals were not captured. Capture of the reproductive female and at least one reproductive male characterized a complete colony (see description below).

Upon capture, animals were assigned a reproductive class (Reproductive, Non-reproductive) based on genitalia and body mass. Prominent teats and a perforate vagina

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identified reproductive females, and only one consistent reproductive female was found in each colony throughout the whole study period. Reproductive males typically have prominent inguinal testes and yellow staining around the mouth (Bennett and Faulkes 2000). However, testes may not always be felt even on large males, and yellow staining around the mouth was not always present in the heaviest males (Tobias Süess and Kyle Finn, University of Pretoria, Pretoria, South Africa, personal communication, September 2021). Thus, we assigned breeding status of males based on both body mass and longitudinal capture data (i.e., repeated presence of the largest males in the colony over multiple captures).

All procedures were carried out according to ASM guidelines (Sikes et al. 2016). The Animal Use and Care Committee of the University of Pretoria evaluated and approved the experimental protocol (ethics clearance NAS040/2019 and EC001-19), and the study received DAFF section 20 approval (SDAH-Epi-20070806200 and SDAH-Epi-21072908241).

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Study site climate.

We retrieved monthly mean climate data from 1981 to 2021 from the global atmospheric reanalysis dataset ERA5-Land (Muñoz-Sabater 2019) for each study site (Table 2). This dataset has a 9 km accuracy (0.1° x 0.1°) and is freely available from the Copernicus Climate Change Service, which holds intellectual property rights of the raw data. The results contain modified Copernicus Climate Change Service information. The datasets obtained were similar to temperatures measured *in situ* at the Natal mole-rat location (Finn et al. 2022). Hence, we assumed a similar level of accuracy at the other two locations. Next, we converted T_{air} and T_{dew} to °C. Then, we calculated mean annual relative humidity (RH) based on the Magnus approximation (Alduchov and Eskridge 1996):

$$RH = 100 \times 10^{7.591386\left(\frac{T_{dew}}{T_{dew} + 240.7263} - \frac{T_{air}}{T_{air} + 240.7263}\right)}$$

The Romanenko estimation (Romanenko 1961) was then used to calculate mean annual potential evapotranspiration (*PET*) using the following formula:

$$PET = 0.00006 \times (100 - RH) \times (25 + T_{air})^2$$

To measure aridity of each study site, we calculated an aridity index (AI) for each year using *PET* and *P* (converted to mm) from the dataset:

$$\mathrm{AI} = \frac{P}{PET}$$

To accurately calculate AI, the mean of many decades is required to account for year-to-year variability in precipitation amounts and aridity conditions (Sahin 2012). As this study attempted to understand whether the AI affects population demographics and body mass (through evolutionary adaptation), we believe it is necessary to report average AI over 40 years that multiple generations would have experienced. Habitats with an AI score < 0.20 are considered arid, between 0.20 and 0.50 semi-arid, and above 0.65 humid or mesic (Colantoni et al. 2015).

A one-way ANOVA test was used to compare the annual AI between study sites. Because *P* was the mean daily rainfall per month, we converted it to mean monthly rainfall by multiplying each value by the number of days in the month. This value was then summed across the rainy season (from July to June of the subsequent year) and divided by the period (i.e. 40 years) to calculate mean annual rainfall for each study site. We then used a series of ANOVA tests with Tukey post-hoc tests to compare climatic variables (T_{air}, $T_{s1\text{-}3}$, $M_{s1\text{-}3}$, and annual rainfall) between study sites. All statistical analyses were performed in R 4.0.3 (R Development Core Team 2021), and means are reported as mean ± *SD*.

Population demographics.

Population demographics measured in this study included colony size, population density, age class, litter size, sex ratio, and colony biomass. Only complete colonies were used to

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analyze population demographics parameters. Population density was calculated as mean number of unique individuals captured per year divided by total area of the study area. Study area is represented as a polygon encompassing all colonies at a location. One-way ANOVA with a Tukey post-hoc test was used to compare population density between subspecies.

Animals were assigned to age classes (juvenile, sub-adult, and adult) based on body mass—where individuals weighing < 40 g were classed as juveniles, 40 – 60 g as sub-adults, and > 60 g as adults (Spinks et al. 2000a; Hart et al. 2021a). The body mass of 40 g was used to separate juveniles from other age classes because examination of anatomy and histology has shown that these individuals are not sexually mature (Spinks et al. 2000a). Age demographics did not differ between seasons (all $P = 0.15$) using complete colonies only. Hence, we calculated proportions of each age class present within each subspecies over the entire study period (i.e., all captures combined) and ran a series of chi-square tests to explore how proportions varied between subspecies. Relatedly, litter size within each colony was estimated by grouping individuals < 40 g into similar-sized juvenile cohorts based on body mass. Grouping by body mass assumes that similar-sized juvenile or sub-adult individuals were born to the same litter and that individuals < 40 g were not immigrants (Spinks et al. 2000b). Non-parametric tests were required to analyze variation in litter size. A Kruskal-Wallis test was used to compare litter sizes between subspecies, followed by post-hoc pairwise Mann-Whitney U-tests for each paired subspecies using complete colonies only.

We then ran a series of generalized linear mixed models (GLMMs) in the package *glmmTMB* (Brooks et al. 2017) to assess differences in colony size, biomass, and sex ratios between subspecies using complete colonies only. Colony size is defined as number of individuals captured within a colony. Biomass is total body mass of all individuals in a complete colony at capture. Finally, we calculated within-colony sex ratios for each subspecies as the mean proportion of all males within each colony (i.e., $N_{males}/N_{females}$ +

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N_{males}), regardless of age or reproductive class (i.e. secondary sex ratio) for each subspecies, using methods of Ancona et al. (2017). Thus, a colony composed of all females receives a score of 0, equal proportions of both sexes a score of 0.5, and a colony of all males a score of 1.

We then fitted a GLMM with colony size in a zero-truncated Poisson distribution as a response variable, subspecies (factor with three levels), and season (factor with two levels: wet/dry) as predictors. Secondly, we fitted biomass in a GLMM with a Gamma distribution as a response variable and species, season, and sex ratio as predictors. Lastly, we fitted sex ratio with a beta distribution as a response variable and species, season, and colony size as predictors. Colony identity was specified as a random intercept in all models to control for non-independence among data of each colony. Post-hoc tests were then run on each subspecies independently to determine effects of each predictor variable (e.g. season, colony size, sex ratio) within each subspecies.

Adult body mass.

We excluded sub-adults and juveniles from body mass analyses because juveniles and sub-adults grow quicker than adults, and body mass correlates strongly with age (Bennett et al. 1991; Thorley et al. 2021). Therefore, we included all body mass values from each adult animal of complete and incomplete colonies at capture for a total sample size of 175 Mahali (males = 93, females = 82), 143 highveld (males = 78, females = 65), and 427 Natal (males = 244, females = 183) mole-rats. We first compared body mass of each sex and reproductive class among subspecies using a GLMM with a Gamma distribution to explore how body mass differed between subspecies. This was done by including subspecies and sex (factor with two levels) and their two-way interaction as fixed predictor variables. Next, we used individual GLMMs with a Gaussian distribution for Mahali and highveld mole-rats and a

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Gamma distribution for Natal mole-rats to determine how body mass was influenced by life history factors (sex and reproductive class), demographics (colony size), or abiotic (season) factors in each subspecies. Again, we used body mass as the response variable and the same fixed predictors as the first model but also included colony size (continuous variable), season, sex*reproductive class, and colony size*season as additional predictors. Finally, individual identity was specified as a random intercept to control for non-independence among data (Supplementary Data SD1).

Results

Study site climate analysis.

Results from the ANOVA tests indicated that study sites possessed significant differences in climatic variables (Table 3; Table 4). These variables were significantly different between seasons where temperatures, rainfall, and soil moisture content were higher during wet season at all sites (Fig. 1; Table 3). Tukey post-hoc tests revealed that Brits and the National Botanical Gardens had similar annual rainfall ($P = 0.48$). During the dry season similar T_{air}: ($P = 0.17$) and T_{s1}: ($P = 0.09$) were observed, but all other mean variables were significantly different between study sites within seasons ($P < 0.05$, Supplementary Data SD2). Glengarry had the lowest T_{air} in both seasons and highest annual rainfall, and Brits had a significantly higher T_{air} during the wet season than the Gardens (Fig. 1; Table 4). The AI scores were similar for Brits (0.38 ± 0.15) and the Gardens (0.43 ± 0.16, $P = 0.64$; Table 1), but Glengarry had a significantly higher AI score (1.88 ± 0.38) compared to the other sites (Brits, $P < 0.001$; Gardens, $P < 0.001$; Table 1).

Population demographics.

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A total of 137 Mahali mole-rats from 19 complete colonies at Brits, 130 highveld mole-rats from 33 complete colonies at the National Botanical Gardens, and 405 Natal mole-rats from 57 complete colonies at Glengarry were used to investigate population demographics of the three subspecies. The three subspecies had an annual population density of 5.5/ha (Mahali mole-rat), 2.2/ha (highveld mole-rat), and 4.9/ha (Natal mole-rat), respectively (Table 1). The population density was significantly different between the highveld and Mahali mole-rats only ($P < 0.05$), while there were no significant differences between the others (Natal and Mahali mole-rat, $P = 0.75$; Natal and highveld mole-rats, $P = 0.07$).

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Age demographics differed among the three subspecies (Fig. 2; Table 1). There was a significantly higher percentage of adults in the highveld mole-rat compared to the Natal mole-rat ($\chi^2(1) = 4.3$, $P = 0.04$) and Mahali mole-rat ($\chi^2(1) = 23.7$, $P < 0.001$). The highest percentage of sub-adults was found in the Mahali mole-rat, significantly higher than the highveld mole-rat ($\chi^2(1) = 6$, $P = 0.01$), but not the Natal mole-rat ($\chi^2(1) = 0.03$, $P = 0.85$). The highest percentage of juveniles was found in the Mahali mole-rat, significantly more so than the highveld mole-rat ($\chi^2(1) = 13.9$, $P < 0.001$) and the Natal mole-rat ($\chi^2(1) = 36.9$, $P < 0.001$). Litter sizes were comparable between the highveld (1.3 $\underline{+}$ 0.5) and Natal mole-rats (1.4 + 0.6), but significantly larger in the Mahali mole-rat (1.9 $\underline{+}$ 0.8) compared to the highveld mole-rat and the Natal mole-rat (Table 1).

Mean colony size was similar between Natal and Mahali mole-rats (est = 1.5, $P = 0.32$), but the highveld mole-rat had significantly smaller colonies compared to the Mahali mole-rat (est = - 3.7, $P = 0.002$) and Natal mole-rat (est = - 2.1, $P = 0.02$, Fig. 3; Table 1). Colony size was not significantly different between seasons in any of the subspecies (highveld mole-rat: est. = -0.32, $SE = 0.20$, $P = 0.11$, Mahali mole-rat: est. = -0.21, $SE = 0.13$, $P = 0.23$, Natal mole-rat: est = 0.08, $SE = 0.12$, $P = 0.49$). Biomass of colonies was similar among the three subspecies (est. = -0.13, df = 3, $P = 0.47$; Table 6) and the two seasons (est.

= -0.00, *SE* = 0.09, *P* = 0.310). Colony biomass was significantly different between seasons in the Natal mole-rat (dry = 504.3 ± 224.0, wet = 580.1 ± 340.6, est = -1.92, *SE* = 0.33, *P* = 0.0001) and the highveld mole-rat (dry = 479.2 ± 276.6, wet = 351.5 ± 205.3, est = -1.74, *SE* = 0.59, *P* = 0.01). Within colony sex ratios were similar among the three subspecies (Mahali mol-rat: 0.46 + 0.2, highveld mole-rat: 0.49 + 0.13, Natal mole-rat 0.47 + 0.13, est. = -0.47, *SE* = 0.27, *P* = 0.08). However, overall colony sex ratios were significantly more female-biased with increasing colony size (est. = -0.04, *SE* = 0.02, *P* = 0.03). We found that season had no effect on sex ratios (all $P \geq 0.13$).

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Adult body mass.

We found significant sexual size dimorphism in body mass across the different subspecies, where males were significantly heavier than females (Fig. 4; Table 5). Reproductive individuals weighed significantly more than non-reproductive individuals, with reproductive males weighing significantly more than reproductive females (Table 5). Non-reproductive males weighed significantly more than non-reproductive females only in the Mahali and Natal mole-rats (Table 5). When comparing body mass among subspecies, the highveld mole-rat had significantly heavier females (females: range = 60 – 145g) than the Mahali mole-rat (females: range = 60 – 112g) and the Natal mole-rat (females: range = 60 – 122g). Male highveld mole-rats were significantly heavier (range = 60 – 179g) than male Natal mole-rats, (range = 60 – 160g), but not significantly different from male Mahali mole-rats (males: range = 60 – 176g, Supplementary Data SD 3). Male and female Mahali mole-rats had a similar body mass compared to male and female Natal mole-rats (Supplementary Data SD4). Reproductive male Mahali mole-rats were significantly heavier than reproductive male Natal mole-rats, but reproductive female Natal mole-rats were heavier than reproductive female

Mahali mole-rats (Supplementary Data SD 4). Non-reproductive males and females exhibited a similar body mass in both Mahali and Natal mole-rats (Supplementary Data SD2). Only Mahali mole-rat individuals were heavier in the dry compared to wet season (Table 6). Body mass was not affected by colony size in any of the subspecies; however, interaction between colony size and season was significant in the Natal mole-rat (Table 6).

Discussion

When considering the evolutionary pathway of the *C. hottentotus* complex through South Africa, the Mahali mole-rat has been considered the most basal and arid-adapted of all members of the *C. hottentotus* species complex (Visser et al. 2019a, 2019b, see Supplementary Data SD1 for proposed evolutionary pathway). As such, according to all current hypotheses (Jarvis et al. 1994; Burda et al. 2000; Hart et al. 2022a), we predicted that the Mahali mole-rat would have retained (Burda et al. 2000) or evolved (Jarvis et al. 1994) larger colony sizes, reduced individual body mass (across both sex and reproductive status), and reduced population density compared to the more mesic-dwelling and mesic-adapted *C. hottentotus* subspecies (Natal, highveld and common mole-rat). As the ancestral Natal, highveld, and common mole-rat moved into more mesic environments (Supplementary Data SD1), there would be an evolutionary selection for reduction in colony size, while body mass (across both sex and reproductive status) and population density would increase (Jarvis et al. 1994; Burda et al. 2000; Hart et al. 2022a). Thus, we would predict that the hyper-mesic-dwelling Natal mole-rat would possess the smallest colony size while possessing the highest body mass (across both sex and reproductive status) and population density, followed by the common mole-rat, with the semi-arid-dwelling highveld mole-rat possessing the largest colony size and colony biomass while possessing the lowest body mass (across both sex and reproductive status) and population density of all three of the mesic-adapted *Cryptomys hottentotus* subspecies (Natal, highveld and common mole-rat).

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As predicted, the Mahali mole-rat, speculated to be the most arid-adapted (and basal) subspecies of the *C. hottentotus* complex (Faulkes et al. 2004; Visser et al. 2019a, 2019b), possessed the largest colony sizes of all subspecies tested (Table 1). Therefore, we hypothesize that evolutionary history of the Mahali mole-rat, which saw this subspecies and its common ancestor being exposed to arid/semi-arid conditions for an extended period of time, resulted in the Mahali mole-rat retaining/evolving larger colonies due to evolutionary fitness benefits put forward in both the AFDH and BOH (Jarvis et al. 1994; Hart et al. 2022a). However, in contrast to what was predicted in this study, the Natal mole-rat (the most mesic-dwelling subspecies) was found to have the second largest colony size, followed by both the mesic and arid populations of common mole-rat, with the highveld mole-rat possessing the smallest colony sizes (Table 1). This finding suggests that factors beyond aridity—such as ambient temperature, soil type, and geophyte availability —might also influence colony size.

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The burrow system of the mole-rat has long been considered a refugium from above-ground temperature extremes, resulting in inhabitants experiencing a muted temperature range (Roper et al. 2001). However, recent research has shown that various mole-rat species experience a wide range of burrow temperatures, close to and sometimes more varied than ambient temperature (Holtze et al. 2018; Finn et al. 2022). Each subspecies of *C. hottentotus* would therefore experience its own unique temperature ranges (Table 1), which may drive evolutionary adaptation—including behavioural thermoregulation via huddling (Kotze et al. 2008; Šumbera et al. 2019). The common, Mahali, and highveld mole-rats may experience soil temperatures ~10°C below their thermal neutral zone (TNZ), but this is only for short periods in the day and at certain times of the year (Table 1). In contrast, the Natal mole-rat—a montane-dwelling mole-rat—regularly experiences soil temperatures of ~10°C and often even ~20°C below their TNZ (Table 1). Hart et al. (2022a) suggested that mesic-dwelling social mole-rat species, such as the Natal mole-rat, may retain a social lifestyle since there is an energetic, thermoregulatory, and osmoregulatory benefit to remaining as a colony-living species. Since the Natal mole-rats at our study site experience the coldest environmental temperatures of all *C. hottentotus* subspecies (Table 1), a larger colony size may have been retained/selected to facilitate effective thermoregulation (huddling; Kotze et al. 2008) even though they (and their likely ancestors) were mesic-adapted (see TNZ in Table 1, see Supplemental Material SD1). The energetic importance of huddling in the Natal mole-rat (Kotze et al. 2008) and other mesic-dwelling social mole-rat species of the genus *Fukomys* has recently been highlighted (Vavrušková et al. 2022). Compared to the Natal mole-rat, a slightly reduced colony size may have been selected for in the common mole-rat due to higher experienced temperatures, which may reduce the need for behavioural thermoregulation and, consequently, larger colonies. Therefore, reduced aridity (compared to the arid-dwelling ancestral *C. hottentotus*) and reduced need for huddling (compared to the Natal mole-rat) may have resulted in reduced colony sizes in the common mole-rat. However, populations of common mole-rats may possess differing physiological thermoregulatory strategies to cope with varying temperature and aridity between sites (Hana Merchant, Royal Holloway University of London, Egham, UK, personal communication, 2022). The highveld mole-rat, likewise, has experienced reduced aridity (compared to the arid-dwelling, ancestral *C. h. mahali*) that would promote reduced colony size, similar to the arid population of the common mole-rat. But unlike both populations of the common mole-rat, and particularly the Natal mole-rat, the highveld mole-rat experiences higher temperatures, reducing the need for larger colonies due to a reduced reliance on behavioural thermoregulation (Table 1).

As such, the combination of exposure to varying temperatures and historical exposure (ancestral exposure) to aridity is likely the driving force behind varying colony sizes in different subspecies of *C. hottentotus* (Table 1). It is likely that all members of the *C. hottentotus* species complex were ancestrally arid-dwelling and may have formed large

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colonies (represented by the Mahali mole-rat); however, as various ancestral populations colonized different habitats across South Africa - with varying levels of aridity and ambient temperatures ranges (and thus a varying need for behavioural thermoregulation) and- these populations diverged into different subspecies adapted to local environmental conditions (e.g., ambient temperature) which has selected for subspecies-specific colony sizes and body masses that are ultimately energetically beneficial. Unfortunately, integration of thermoregulation, including and not limited to huddling, has not been successfully included into the AFDH and the evolution of sociality in African mole-rats. Further studies of soil hardness, geophyte availability, and thermoregulatory benefits in other populations of *Cryptomys* mole-rats across their range are now critically required to test this hypothesis further.

Increased recruitment rates may also support increased colony sizes observed in both Mahali and Natal mole-rats. Both the Natal and Mahali mole-rats breed throughout the year (Oosthuizen et al. 2008; Hart et al. 2021a), whereas the highveld and common mole-rat are seasonal breeders (Spink et al. 1999b; Janse van Rensburg et al. 2002). The Mahali mole-rats may have up to eight pups in a litter (Hart et al. 2019), while the highveld mole-rats have a maximum of three pups per litter and Natal and common mole-rats a maximum of five pups per litter (Janse van Rensburg et al. 2002; Oosthuizen et al. 2008; Bennett and Faulkes 2000; this study). Consequently, the Mahali mole-rat would have higher annual recruitment of colony members than common, Natal, or highveld mole-rats—as evidenced by the higher proportion of juveniles (Table 1). However, differing recruitment rates (differing litter size, and offspring survival rates) between subspecies are likely the result of the differing colony size of subspecies and not the other way around (Houslay et al. 2020). Hart and Bennett (2023) have recently hypothesized that mole-rat species with larger colony sizes (as seen in the Mahali and Natal mole-rat) exhibit an aseasonal pattern of reproduction and increased

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litter size and pup survival because breeders, particularly females, possess year-round high body conditions, likely due to access to abundant and high-quality resources (food and water) throughout the year due to increased efficiency of locating and retrieval of resource as a function of colony size. Alternatively, in social species with smaller colony sizes—such as the highveld mole-rat and, to some extent, the common mole-rat, have fewer colony members to provide sufficient cooperative care benefits, breeders show a seasonality to their body condition and thus breeding investment and litter sizes (Hart and Bennett 2023).

As predicted, as mean colony size increased across subspecies, individual mole-rat body mass decreased (Table 1; Jarvis et al. 1994); however, the apparent outlier is the arid-dwelling common mole-rat population which possessed the lowest body masses of all tested subspecies but the second lowest colony size (Table 1). Reduced body mass translates into substantial energetic savings for colonies in arid environments, potentially enhancing colony and individual survival (Spinks et al. 2000a). The AFDH predicts that when inhabiting an arid environment where food is limited, individual body mass rather than number of animals in a colony may be reduced (Jarvis 1985; Spinks et al. 2000a). However, the opposite trend is observed in the semi-arid-dwelling highveld mole-rat, which possessed the highest mean body mass and smallest colony size likely due to increased food availability and softness of the soil (Spinks et al. 2000a), along with added benefits of colony living (Hart et al. 2022a), resulting in higher population/colony density and colony biomass (Table 1). Unlike the two common mole-rat populations in Spinks et al. (2000a), food resource biomass data were not collected for the Natal, Mahali, and highveld mole-rat populations. As such, we do not wish to speculate on possible causes behind their observed population/colony density and biomass. However, Natal, Mahali, and highveld mole-rat populations have similar high biomass, even compared to their arid- or mesic-dwelling common mole-rat population counterparts (Table 1). While the Natal, Mahali, and highveld mole-rat populations showed higher

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population/colony density than the arid-dwelling common mole-rat population, they exhibited a lower population/colony density than the hyper-mesic-dwelling common mole-rat population (Table 1).

As reported by Spinks et al. (2000a), reproductive individuals of the Natal, Mahali, and highveld mole-rats were found to be the heaviest individuals in their respective sex in the colony. This is likely due to various endocrinological (namely sex hormone differences such as testosterone and progesterone) differences between reproductive and non-reproductive individuals, which likely drive body mass differences—a common trend across all social mole-rat species (Lutermann et al. 2013; Janse van Vuuren 2022; Hart et al. 2022b; Wallace et al. 2023).

In conclusion, this study sought to investigate the link between colony size and biomass in African mole-rats by evaluating the link between colony size and habitat conditions within a social species complex of African mole-rats. In the past, habitat conditions generally referred to rainfall and aridity, which directly affect the distribution, quantity, and quality of food resources. However, this current study adds temperature as another important environmental condition to consider. The effect of ambient temperature as a selection pressure for colony living has likely been neglected because it has long been considered that exclusively subterranean mammals, such as African mole-rats, experience a muted temperature range in their burrow system when compared to surface temperatures. However, recent studies on physiological and behavioural responses of African mole-rats to changes in temperature (van Jaarsveld et al. 2019; Hart et al. 2021b; Wallace et al. 2021; Finn et al. 2022) have revealed that temperature is likely a strong selection pressure acting on African mole-rats. Therefore, the additional selection pressure for increased colony sizes is likely to allow for maximizing foraging efficiency (to increase access to food and water) while minimizing individual energy expenditure of foraging and thermoregulation in colder

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environments through the inexpensive energy strategy of huddling. As such, colony-living likely arises to help the individual to remain in a positive energy balance. However, as these are novel speculations, more longitudinal studies on different populations of the *Cryptomys* subspecies and other poorly studied species of *Fukomys* are needed to investigate various pathways that may affect the energy balance of an individual and the colony as a whole.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

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TS, KF, DH, NB conceived the study. TS, KF, AJvV collected data. TS, KF analyzed the data. TS, KF wrote the first draft of the manuscript and all authors contributed substantially to revisions.

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Data Availability

All data will be made available on request.

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Supplementary Data

Supplementary Data are available at *Journal of Mammalogy* online.

Supplementary Data SD1. —Proposed evolutionary pathway of the *Cryptomys hottentotus* species complex

Supplementary Data SD2.—Results from Tukey post-hoc tests comparing climate variables between study sites within each season.

Supplementary Data SD3.—Post-hoc analyses comparing body mass between sex and reproductive status within and among subspecies.

Supplementary Data SD4.—Post-hoc analyses comparing body mass by sex among subspecies.

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Figure Legends

Fig. 1.—Mean annual climate from 1981 – 2021 at the study locations: Brits (grey), the Pretoria National Botanical Gardens (dark grey), Glengarry (light grey) showing seasonal variation in A) ambient temperature, B) soil temperature (0 – 7 cm), C) soil temperature (7 – 28 cm), D) soil temperature (28 – 100 cm), E) annual rainfall, F) soil moisture (0-7cm), G) soil moisture (7-27cm), and H) soil moisture (28-100cm). The warm wet season occurred from December to May and the cool dry season from June to November. The climate data were obtained from the ERA5-Land dataset supplied by the Copernicus Climate Change Service. The boxes represent median and 25/75% quartiles, with whiskers extending 1.5x beyond interquartile range and outliers as black circles.

Fig. 2.—Age class demographics for *Cryptomys hottentotus mahali*, *C. h. pretoriae*, and *C. h. natalensis*. Mole-rats were grouped into age classes based on body mass: juveniles (grey) <40g, sub-adults (dark grey) 40-60g, and adults >60g (light grey). Proportions were calculated as the number of each age class out of the total population. The numbers in the boxes refer to the sample size of each age class.

Fig. 3.—Group size frequency (A) and mean group size (B) in three subspecies of *Cryptomys hottentotus* (*C. h. mahali* dark grey, *C. h. pretoriae* grey, and *C. h. natalensis* light grey). The

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boxes represent median and 25/75% quartiles, with whiskers extending 1.5x beyond interquartile range and outliers as black circles.

Fig. 4.--Adult body mass in (A) reproductive and (B) non-reproductive individuals in *Cryptomys hottentotus* (*C. h. mahali* dark grey, *C. h. pretoriae* grey, and *C. h. natalensis* light grey). The boxes represent median and 25/75% quartiles, with whiskers extending 1.5x beyond the interquartile range.

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Tables

Table 1.—Summary of population demographics, ecology, and physiology for subspecies of the Hottentot mole-rat (*Cryptomys hottentotus*). Means are given as mean ± SD, except for Spinks et al. (2000b) and Jacob et al. (2022), which is mean ± SE. Numeric values without a reference are from the current study. T_{air} is the mean annual ambient air temperature. RMR is resting metabolic rate. TNZ is thermal neutral zone. SSD is sexual size dimorphism and calculated by dividing the mean mass of males by the mean mass of females.

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	Mahali	Highveld	Natal	Common	Common
Habitat	bushveld	grassland	grassland	Karoo	fynbos, grassland
Annual Rainfall (mm)	635 ± 144.1	677 ± 143.1	1577 ± 211.1	145 ± 9[a]	652 ± 17 [a]
Aridity Score (Min-Max)	0.38 ± 0.15 (0.14-0.91) “Semi-arid” [1]	0.43 ± 0.16 (0.17-1.10) “Semi-arid” [1]	1.88 ± 0.38 (0.77-2.10) “Hyper-mesic” [1]	0.08 ± 0.01[j] (0.03-0.18) “Arid”[1]	0.95 ± 0.03[j] (0.61-1.45) “Hyper-mesic” [1]
Mean T_{air} (°C)	18.9 ± 4.15	18.3 ± 3.38	12.9± 3.78	15.2 ± 3.41	17.6 ± 4.07
T_{air} Range (°C)	10.0 – 26.0	9.7 – 24.7	2.8 – 18.5	9.0 – 25.1	9.4 - 22.5
Breeding system	aseasonal[b]	seasonal[c]	aseasonal[d]	seasonal	seasonal
Breeding Period	Jan - Dec[b]	July – Nov[c]	Jan - Dec[d]	Sept – Nov[e]	Sept – Nov[e]
Reproductive Suppression	Behavioral/ physiological	Behavioral/ physiological	Behavioral	Behavioral	Behavioral
Ovulation	induced	induced	induced	induced	induced
RMR (ml O_2 g^1 h^{-1})	1.19 – 1.23[f]	0.68[f]	0.80 – 1.03[f]	0.92 – 1.38[f]	-

Body temp Range (°C)	34.8 – 35.2^{f}	35.8^{f}	32.2 – 36.1^{g}	34.4^{f}	-
	homeothermic	homeothermic	heterothermic	homeothermic	homeothermic
TNZ (°C)	28 – 36^{f}	30 – 32^{f}	30 – 31.5^{f}	27 – 30^{f}	-
Annual Population density (individuals/hectare * year)	5.5 ± 1.1	2.2 ± 0.0	4.9 ± 0.7	1.8 ± 0.2^{a}	18.7 ± 1.2^{a}
Colony density (colonies/hectare)	1.5	1.2	1.2	1.8	16.0
Colony size	7.7 ± 5.2	4.0 ± 2.7	6.6 ± 2.9	5.1 ± 0.2^{a}	5.1 ± 0.2^{a}
Juveniles [% of total population]	45 [15.9%]	10 [5.7%]	28 [6.6%]	39 [11.6 %]^{h}	57 [12.6%]^{h}
Sub-adults	65	22	99	-	-
Adults	175	143	295	295^{h}	393^{h}
Sex Ratio	0.46 ± 0.19	0.49 ± 0.14	0.47 ± 0.13	0.54^{h}	0.69^{h}
Biomass (g)	509.7 ± 315.7	410.8 ± 245.1	535.3.7 ± 272.6	286.9 ± 12.3^{a}	382.6 ± 15.7^{a}
Litter Size	1.9 ± 0.8	1.3 ± 0.5	1.4 ± 0.6	2.6 ± 0.2^{h}	2.3 ± 0.2^{h}
Mean body mass (g)	66.9 ± 33.7	101.1 ± 35.2	78.6 ± 26.0	57.8± 0.8^{a}	75.2 ± 1.0^{a}
Maximum	173	179	160	94	132
Male	104.3 ± 27.8	120.5 ± 30.6	93.1 ± 22.1	-	83 ± 22.7^{i}
Female	75.7 ± 11.9	98.7 ± 19.8	80.3 ± 14.1	-	58 ± 14.6^{i}
SSD	1.38	1.22	1.16		1.43

[a] Spinks et al. 2000b; [b] Van der Walt et al. 2001; Hart et al. 2021, Hart et al. 2022; [c] Janse van Renseburg et al. 2002; [d] Oosthuizen et al. 2008; [e] Spinks et al. 2000a; [f] Šumbera et al. 2019; [g] Oosthuizen et al. 2021; [h] Spinks et al. 2000c; [i] Davies and Jarvis 1986; [j] Jacobs et al. 2022; [k] Wallace et al. 2021; [l] Climate definitions set out by Colantoni et al. (2015).

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Table 2.—Variables from ERA5-Land monthly dataset used for climate analysis at 3 sites inhabited by *Cryptomys hottentotus* subspecies. The data are freely available from the Copernicus Climate Change Service which holds the intellectual property rights of the raw data. The given units are the units provided in the dataset.

Variable	Description	Units
2m temperature (T_{air})	The temperature of the air 2m above the soil surface	K
2m dewpoint temperature (T_{dew})	Temperature to which the T_{air} would have to be cooled for saturation to occur; used to measure the humidity of the air	K
Total precipitation (P)	The daily mean of accumulated liquid and frozen water that falls to the Earth’s surface	m
Soil temperature level 1 (T_{s1})	The temperature of the soil in layer 1 (depth 0 – 7cm), taken from the middle of each layer	K
Soil temperature level 2 (T_{s2})	The temperature of the soil in layer 2 (7 – 28cm)	K
Soil temperature level 3 (T_{s3})	The temperature of the soil in layer 3 (28 – 100cm)	K
Volumetric soil water layer 1 (M_{s1})	The volume of water in soil layer 1 (0 – 7cm); a measure of soil moisture	m^3m^{-3}
Volumetric soil water layer 2 (M_{s2})	The volume of water in soil layer 2 (7 – 28cm)	m^3m^{-3}
Volumetric soil water layer 3 (M_{s3})	The volume of water in soil layer 3 (28 – 100cm)	m^3m^{-3}

Table 3.—Results from ANOVA tests for climate differences between study sites. See Table 1 for abbreviations. Rain refers to mean annual rainfall. Aridity was calculated as mean monthly rainfall divided by mean potential evapotranspiration.

	DF	Sum Sq	*F*-value	*P*-value
T_{air}	2	227.9	987.8	< 0.001
T_{s1}	2	8122.3	1152.2	< 0.001
T_{s2}	2	8079.0	1313.9	< 0.001
T_{s3}	2	7999.6	1669.6	< 0.001
Rain	2	51.9	392.4	< 0.001
M_{s1}	2	7.5	4642.2	< 0.001
M_{s2}	2	622.0	5467.3	< 0.001
M_{s3}	2	667.1	8871.7	< 0.001
Aridity	2	60.2	480.7	< 0.001

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Table 4.—Mean climate variables by season for 3 study sites where subspecies of the Hottentot mole-rat were captured (Brits: Mahali mole-rat, *C. h. mahali*, Pretoria National Botanical Gardens: Highveld mole-rat, *C. h. pretoriae*, Glengarry: Natal mole-rat *C. h. natalensis*). The dry season is from April – September, and the wet season is from October – May. Rainfall is the mean seasonal rainfall for each season. The mean annual values (± SD) for each variable were derived from the ERA5-Land dataset from 1981 to 2020. Tukey post-hoc tests indicated all values at a study site were highly significant between seasons (Supplementary Data SD1). See Table 1 for abbreviations.

	Brits		Gardens		Glengarry	
	Dry	Wet	Dry	Wet	Dry	Wet
T_{air} (°C)	15.3 ± 2.98	22.0 ± 1.55	14.8 ± 2.9	21.1 ± 1.4	9.6 ± 2.66	15.8 ± 1.51
T_{s1} (°C)	17.2 ± 3.41	24.8 ± 2.04	16.6 ± 3.3	23.7 ± 1.9	9.8 ± 3.13	17.3 ± 1.54
T_{s2} (°C)	17.4 ± 3.13	24.5 ± 1.91	16.8 ± 3.0	23.4 ± 1.7	10.1 ± 2.97	17.0 ± 1.56
T_{s3} (°C)	18.3 ± 2.63	23.7 ± 1.70	17.5 ± 2.5	22.7 ± 1.6	11.0 ± 2.67	16.1 ± 1.70
Rain (mm)	95.5 ± 50.32	537.7 ± 133.3	101.8 ± 55.68	575.7 ± 124.7	316.5 ± 104.0	1260.9 ± 181.1
M_{s1} (m^3 m^{-3})	0.22 ± 0.04	0.29 ± 0.05	0.18 ± 0.04	0.25 ± 0.05	0.43 ± 0.03	0.46 ± 0.01
M_{s2} (m^3 m^{-3})	0.24 ± 0.03	0.28 ± 0.05	0.18 ± 0.04	0.23 ± 0.05	0.43 ± 0.03	0.47 ± 0.01
M_{s3} (m^3 m^{-3})	0.24 ± 0.03	0.24 ± 0.04	0.18 ± 0.03	0.18 ± 0.04	0.43 ± 0.03	0.47 ± 0.03

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Table 5.—Comparison of adult body mass of Common mole-rat (*Cryptomys hottentotus*) subspecies between reproductive and non-reproductive individuals of both sexes. SSD is sexual size dimorphism, the mass of males divided by the mass of females.

	Mean Body Mass (g)		SSD	Df	P-values		
	Males	Females			*mahali*	*pretoriae*	*natalensis*
Mahali ($n = 140$)	104.3 ± 27.8	75.7 ± 11.9	1.38	1	< 0.001	-	-
Highveld ($n = 172$)	120.5 ± 30.6	98.7 ± 19.8	1.22	1	-	0.67	-
Natal ($n = 427$)	93.1 ± 22.1	80.3 ± 14.1	1.16	1	-	-	< 0.001
Non-reproductive:							
Mahali ($n = 75$)	83.9 ± 17.6	72.6 ± 10.2	1.16	1	0.004	-	-
Highveld ($n = 89$)	93.5 ± 21.3	90.3 ± 16.6	1.04	1	-	1.00	-
Natal ($n = 266$)	84.6 ± 17.0	75.5 ± 12.1	1.12	1	-	-	< 0.001
Reproductive:							
Mahali ($n = 65$)	122.3 ± 22.1	80.6 ± 12.9	1.52	1	< 0.001	-	-
Highveld ($n = 83$)	138.9 ± 20.6	114.2 ± 15.7	1.22	1	-	0.008	-
Natal ($n = 121$)	108.7 ± 21.9	91.8 ± 11.9	1.18	1	-	-	< 0.001

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Table 6.—Model output from all general linear mixed model comparing body mass among *Cryptomys hottentotus* subspecies. Blanks indicate that the removal of the terms provided a better fit for the model.

	All Species			Mahali			Highveld			Natal		
Fixed Effect	Est	SE	*P*-value	Est	SE	*P*-value	Est	SE	*P*-value	Est	SE	*P*-value
Intercept	4.32	0.03	< 0.001	4.32	0.06	< 0.001	93.79	5.21	< 0.001	77.96	2.62	< 0.001
Species	See text			-	-	-	-	-	-	-	-	-
Sex	0.29	0.04	< 0.001	0.15	0.04	< 0.001	2.27	5.38	0.674	9.99	2.70	< 0.001
Reproductive class	-	-	-	-	-	-	-	-	-	-	-	-
Season	-	-	-	-0.09	0.03	0.003	- 4.26	2.77	0.119	-9.37	3.75	0.012
Colony Size	-	-	-	0.00	0.00	0.686	- 0.25	0.52	0.635	-0.24	0.27	0.367
Sex*Reproductive class	-	-	-	0.12	0.05	0.018	24.67	5.21	< 0.001	7.69	4.81	0.112
Season*Colony Size	-	-	-	-	-	-	-	-	-	0.95	0.37	0.011

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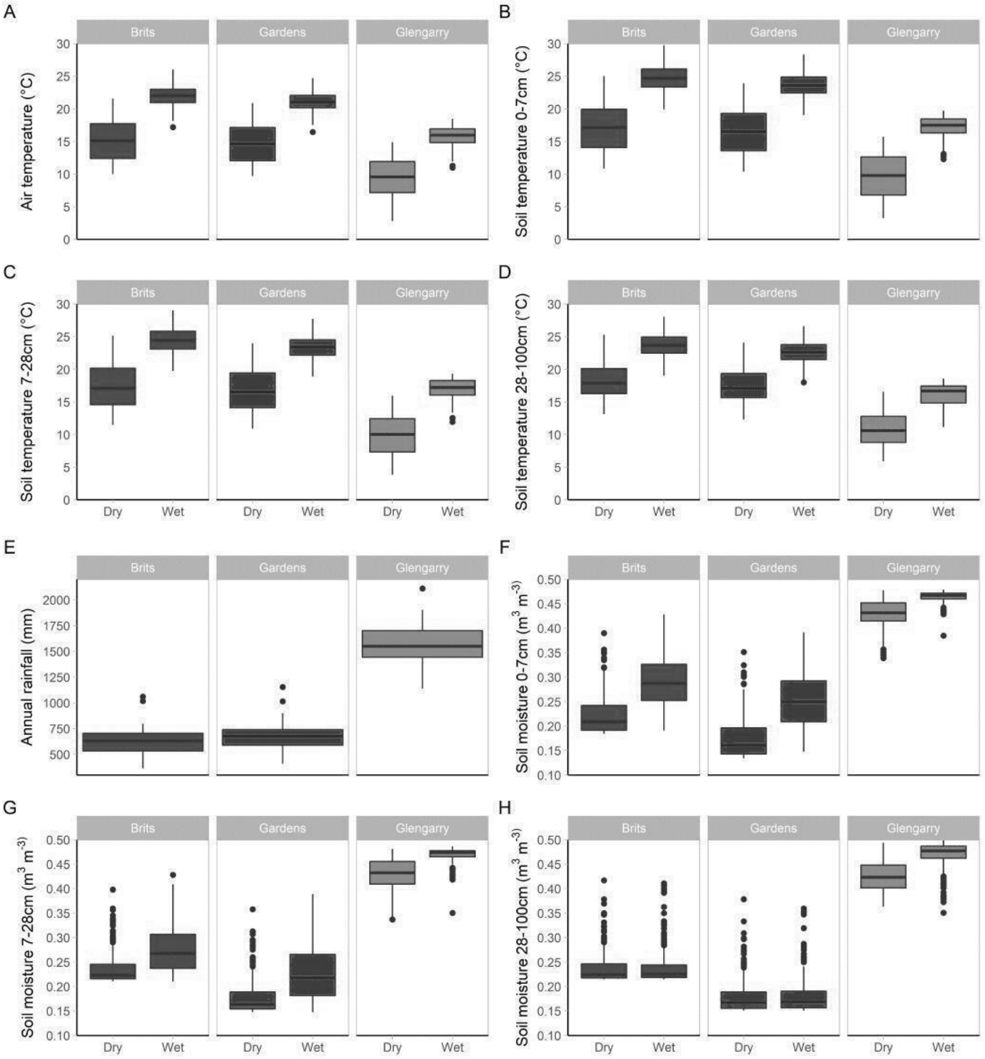

Fig. 1: Mean annual climate from 1981 – 2021 at the study locations: Brits (grey), the Pretoria National Botanical Gardens (dark grey), Glengarry (light grey) showing seasonal variation in A) ambient temperature, B) soil temperature (0 – 7 cm), C) soil temperature (7 – 28 cm), D) soil temperature (28 – 100 cm), E) annual rainfall, F) soil moisture (0-7cm), G) soil moisture (7-27cm), and H) soil moisture (28-100cm). The warm wet season occurred from December to May and the cool dry season from June to November. The climate data were obtained from the ERA5-Land dataset supplied by the Copernicus Climate Change Service. The boxes represent median and 25/75% quartiles, with whiskers extending 1.5x beyond interquartile range and outliers as black circles.

45x48mm (600 x 600 DPI)

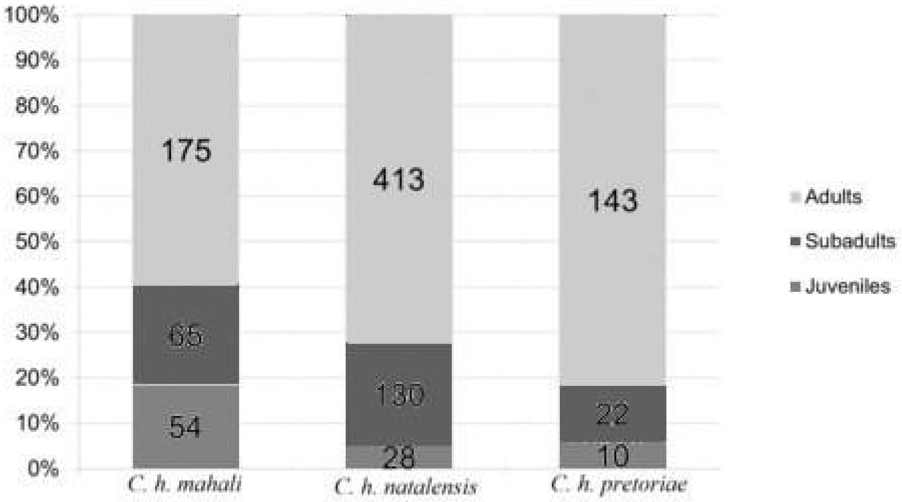

Fig. 2: Age class demographics for C. h. mahali, C. h. pretoriae, and C. h. natalensis. Mole-rats were grouped into age classes based on body mass: juveniles (grey) <40g, sub-adults (dark grey) 40-60g, and adults >60g (light grey). Proportions were calculated as the number of each age class out of the total population. The numbers in the boxes refer to the sample size of each age class.

21x12mm (600 x 600 DPI)

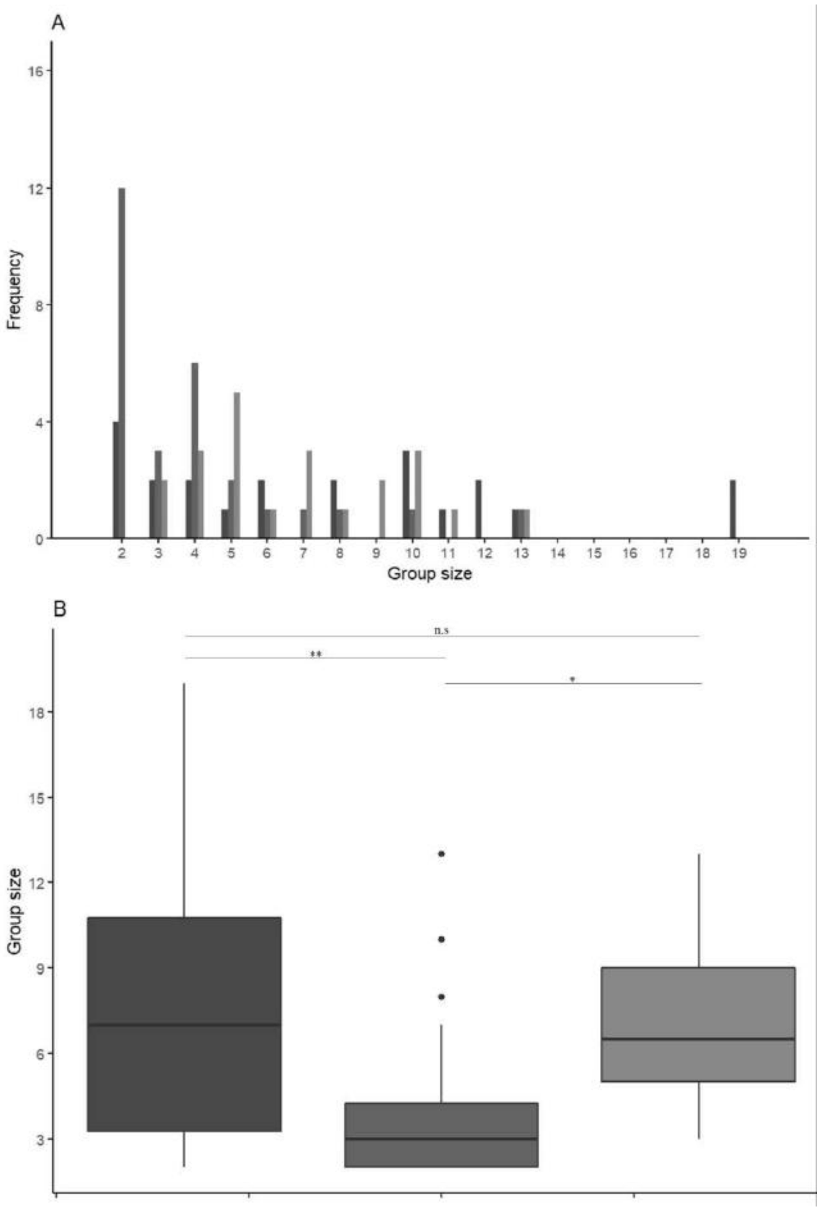

Fig. 3: Group size frequency (A) and mean group size (B) in three subspecies of Cryptomys hottentotus (mahali dark grey, pretoriae grey, and natalensis light grey). The boxes represent median and 25/75% quartiles, with whiskers extending 1.5x beyond interquartile range and outliers as black circles.

83x122mm (600 x 600 DPI)

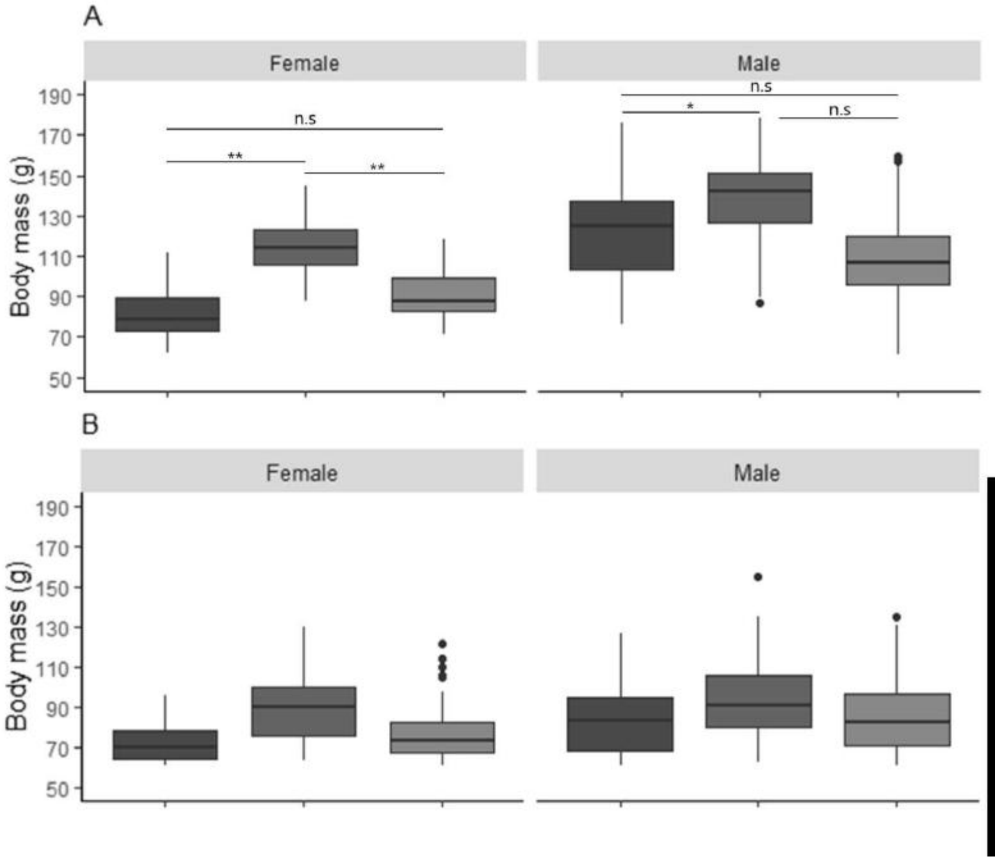

Fig. 4: Adult body mass in (A) reproductive and (B) non-reproductive individuals in Cryptomys hottentotus (mahali dark grey, pretoriae grey, and natalensis light grey). The boxes represent median and 25/75% quartiles, with whiskers extending 1.5x beyond the interquartile range.

91x78mm (600 x 600 DPI)

Supplementary Data D1. —Proposed evolutionary pathway of *Cryptomys hottentotus* complex.

There are currently five recognized subspecies of *Cryptomys hottentotus*; four of the five subspecies are endemic to South Africa, namely the common mole-rat (*C. h. hottentotus*), the Natal mole-rat (*C. h. natalensis*), the highveld mole-rat (*C. h. pretoriae*), the Mahali mole-rat (*C. h. mahali*), and one subspecies, the Matabeleland mole-rat (*C. h. nimrodi*), endemic to Botswana and Zimbabwe (Faulkes et al. 2004). To date, no subspecies have been recorded to occur with one another sympatrically. As *Cryptomys hottentotus* dispersed across southern Africa, a significant divergence between the different subspecies of *Cryptomys hottentotus*, which had split from the common ancestor in the mid to late Miocene (~18 million years ago [mya]) occurred (Faulkes et al. 2004; Visser et al. 2019a, 2019b). Recent studies have shown a significant divergence between the populations of *Cryptomys hottentotus* and may be distinct species (Faulkes et al. 2004; Merchant et al. unpublished data; Visser et al. 2019a; Visser et al. 2019b). However, due to the lack of any formal descriptions to elevate the subspecies of *Cryptomys hottentotus* to full specific status, we refer to them here as subspecies.

As it stands, there is no consensus regarding the evolutionary history of the *Cryptomys hottentotus* complex; however, it is speculated that the common ancestor of *Cryptomys hottentotus* first invaded arid environments of northern and then central South Africa, which either drove group-living [according to Jarvis et al. (1994)] or maintained colony living [according to Burda et al. (2000)] and ultimately moved south towards the coast (see Faulkes

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et al. 2004). These ancestral populations were unable to inhabit all areas of South Africa, including the South African highveld (Johannesburg and South Pretoria, South Africa), due to physical barriers, such as the Magaliesberg mountain range, which prevented these ancestral populations from moving from the north to the south of Pretoria, South Africa (Visser et al. 2019a). The population of the *Cryptomys hottentotus* common ancestor that remained in these areas of northern and central South Africa, including those north of the Magaliesberg mountain range and those that reach the south coast of South Africa (see Visser et al. 2019a), likely gave rise to the Mahali mole-rat subspecies complex; the speculated basal lineage of *Cryptomys hottentotus* complex (Faulkes et al. 2004; Visser et al. 2019a). This would explain the current distribution of the Mahali mole-rat, including the population found on the southern coast of South Africa (see Visser et al. 2019a). Upon reaching the southern coast of South Africa, the ancestral *Cryptomys hottentotus* complex moved both east and west into less arid (mesic) environments, ultimately giving rise to the common mole-rat complex, the speculated next step in the *Cryptomys hottentotus* complex lineage (Faulkes et al. 2004; Visser et al. 2019a). The common mole-rat rapidly inhabited, firstly, the hyper-mesic western coast (south to north) of South Africa and then rapidly moved north and slightly into the interior into hyper-arid environments in the last ~4 million years (Faulkes et al. 2004; Visser et al. 2019a). As the *Cryptomys hottentotus* complex moved east from the southern coast of South Africa, they inhabited the hyper-mesic eastern face of the Drakensberg mountain range (Visser et al. 2019a) but, to date, were unable to summit the mountain range. Upon moving north along the eastern face of the Drakensberg mountain range, they were likely able to move inland towards South African Highveld (Johannesburg and southern Pretoria, South Africa) through areas of reduced elevation of the Drakensberg mountain range (~ 100 km north of Wakkerstroom, South Africa) (See Visser et al. 2019a). Those populations that have

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remained on the eastern face of the Drakensberg mountain range ultimately gave rise to the Natal mole-rat (Visser et al. 2019a), while the population that moved further inland into the South African Highveld (Johannesburg and southern Pretoria, South Africa) gave rise to the highveld mole-rat. The Natal and highveld mole-rat are closely related to one another (sister clades) and are the most recent diverged clade of the *Cryptomys hottentotus* complex (Visser et al. 2019a).

As such the Mahali mole-rat, representing the basal lineage of *Cryptomys hottentotus* complex, has been considered the most arid-adapted of all *Cryptomys hottentotus* subspecies, with many physiological adaptations supporting this, including thermoregulatory adaptations, such as the breadth of the mammal thermoneutral zone (TNZ) (Table 1). The breadth of a mammal TNZ, the range of ambient temperatures that require minimal metabolic regulation to maintain body temperature, has been found to correlate with the mammal's habitat aridity, namely mammals that inhabit arid regions possess longer TNZ to aid in increased water and energy savings (Sumbera 2019; Wallace et al. 2021). The Mahali mole-rats possess the longest thermoneutral zone (TNZ) of all *Cryptomys hottentotus* subspecies (~8°C, Table 1). Conversely, all other *Cryptomys hottentotus* subspecies possess equally short TNZs (~2°C, Table 1).

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Model selection & validation

Statistical models

The models had to account for a zero-truncated distribution because each group counts at least two individuals. We used stepwise deletion of the fixed effect predictors to find the model with the lowest AIC value.

We first ran a GLMM in the package nlme, visually inspected the residuals, plotted them against fitted values (Zuur, 2009), and ran a Shapiro test. Values of body mass were normally distributed and independent for *C.h. mahali* and *C.h. pretoriae* only (*C.h. mahali*, p = 0.29; C.h. *pretoriae*, p = 0.44). However, values of residuals for the model with *C.h. natalensis* and all three mole-rats together followed a skewed distribution (Shapiro-Test: *C.h. natalensis*, p = 0.007; all subspecies, p < 0.001 **). We, therefore, ran all models as a GLMM, some with a Gamma distribution and a log link and some with a Gaussian distribution. We used the *emmeans* package (Lenth et al. 2018) to get p-values for interactions and categorical variables with at least three levels and specified the Bonferroni correction for multiple comparisons.

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Random effects

The inclusion of individual identity as a random effect for the body mass models instead of group identity provided a better fit for the models (all subspecies AIC: individual 5043.5, individual+group no convergence, Mahali mole-rats AIC: individual 1072.3, individual+group no convergence; highveld mole-rats AIC: individual 1079.7, individual+group no convergence; Natal mole-rats AIC: individual 830.2, individual+group no convergence).

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Supplementary Data SD2.—Results from Tukey post-hoc tests comparing climate variables between study sites within each season.

Supplementary Data Table D2.—Results from Tukey post-hoc tests comparing climate variables between study sites within each season. * marks significance

	95% CI	p-value
T_{air}		
BritsDry-GardensDry	-0.23, 0.02	0.179
BritsWet-GardensWet	-0.32, -0.07	0.002*
BritsDry-GlengarryDry	-1.33, -1.08	<0.001*
BritsWet-GlengarryWet	-1.43, -1.18	<0.001*
GardensDry-GlengarryDry	-1.22, -0.98	<0.001*
GardensWet-GlengarryWet	-1.24, -0.99	<0.001*
T_{s1}		
BritsDry-GardensDry	-1.32, 0.05	0.086
BritsWet-GardensWet	-1.81, -0.44	< 0.001*
BritsDry-GlengarryDry	-8.11, -6.74	<0.001*
BritsWet-GlengarryWet	-8.23, -6.85	<0.001*
GardensDry-GlengarryDry	-7.48, -6.12	<0.001*
GardensWet-GlengarryWet	-7.10, -5.73	<0.001*
T_{s2}		
BritsDry-GardensDry	-1.31, -0.03	0.035*
BritsWet-GardensWet	-1.74, -0.45	< 0.001*
BritsDry-GlengarryDry	-8.03, -6.75	< 0.001*
BritsWet-GlengarryWet	-8.19, -6.90	< 0.001*
GardensDry-GlengarryDry	-7.36, -6.08	< 0.001*
GardensWet-GlengarryWet	-7.09, -5.81	< 0.001*
T_{s3}		
BritsDry-GardensDry	-1.32, -0.19	0.002*
BritsWet-GardensWet	-1.59, -0.45	< 0.001*
BritsDry-GlengarryDry	-7.85, -6.72	< 0.001*
BritsWet-GlengarryWet	-8.15, -7.01	>0.001*
GardensDry-GlengarryDry	-7.10, -5.97	<0.001*
GardensWet-GlengarryWet	-7.12, -5.99	<0.001*
Rainfall		
BritsDry-GardensDry	-1.31, -0.03	0.035*
BritsWet-GardensWet	-1.74, -0.45	< 0.001*
BritsDry-GlengarryDry	-8.03, -6.75	< 0.001*
BritsWet-GlengarryWet	-8.19, -6.90	< 0.001*
GardensDry-GlengarryDry	-7.36, -6.08	< 0.001*
GardensWet-GlengarryWet	-7.09, -5.81	< 0.001*
M_{s1}		
BritsDry-GardensDry	-0.06, -0.04	< 0.001*
BritsWet-GardensWet	-0.05, -0.03	< 0.001*

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BritsDry-GlengarryDry	0.23, 0.25	< 0.001*
BritsWet-GlengarryWet	0.16, 0.18	< 0.001*
GardensDry-GlengarryDry	0.24, 0.26	< 0.001*
GardensWet-GlengarryWet	0.20, 0.22	< 0.001*
M_{s2}		
BritsDry-GardensDry	-0.60, -0.43	< 0.001*
BritsWet-GardensWet	-0.52, -0.35	< 0.001*
BritsDry-GlengarryDry	1.59, 1.76	< 0.001*
BritsWet-GlengarryWet	1.59, 1.77	< 0.001*
GardensDry-GlengarryDry	2.10, 2.28	< 0.001*
GardensWet-GlengarryWet	2.02, 2.20	< 0.001*
M_{s3}		
BritsDry-GardensDry	-0.56, -0.42	< 0.001*
BritsWet-GardensWet	-0.55, -0.41	< 0.001*
BritsDry-GlengarryDry	1.50, 1.64	< 0.001*
BritsWet-GlengarryWet	1.85, 1.99	< 0.001*
GardensDry-GlengarryDry	1.98, 2.13	< 0.001*
GardensWet-GlengarryWet	2.33, 2.47	< 0.001*

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Supplementary Data SD3.—Post-hoc analyses comparing body mass between sex and reproductive status within and among subspecies.

Supplementary Data D3. —Post-hoc analyses of GLMM models comparing body mass of males and females within and between reproductive classes (reproductive vs. non-reproductive) for all subspecies combined, then each of the subspecies (*C.h.natalensis, C.h.pretoriae*; *C.h.mahali*) separately. Models estimate marginal means, including Bonferroni correction from the *emmeans* package in R. RM = reproductive male, RF = reproductive female, NRM = non-reproductive male, NRF = non-reproductive female. * marks significance level

Subspecies	Class	Df	t or z-value	P-value
All	RM (n=154) vs. RF (n=92)	21	-9.479	< 0.001 **
All	NRM (172) vs. NRF (181)	21	-4.72	< 0.001 **
All	Male (326) vs. Female (273)	21	-8.59	< 0.001 **
All	RM (154) vs. NRM (172)	21	-15.906	< 0.001 **
All	RF (92) vs. NRF (181)	21	-7.845	< 0.001 **
All	Reproductive (246) vs. non-reproductive (353)	21	2.269	0.023 *

Subspecies	Class	Df	t-ratio	P-value
C.h.p	RF (28) vs. NRF (46)	123	-5.064	< 0.001 **
C.h.p	RM (50) vs. RF (28)	123	-3.293	0.008 *
C.h.p	NRM (32) vs. NRF (46)	123	-0.303	1
C.h.p	RM (50) vs. NRM (32)	123	-7.851	< 0.001 **
C.h.m	RF (28) vs. NRF (35)	118	-2.375	0.11
C.h.m	RM (50) vs. RF (28)	118	-7.790	< 0.001 **
C.h.m	NRM (30) vs. NRF (35)	118	-3.514	0.004 *
C.h.m	RM (31) vs. NRM (30)	118	-9.050	< 0.001 **
C.h.n	RF vs. NRF	363	-5.923	< 0.001 **
C.h.n	RM vs. RF	363	-7.790	< 0.001 **
C.h.n	NRM vs NRF	363	-4.942	< 0.001 **
C.h.n	RM vs. NRM	363	-9.001	< 0.001 **

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Supplementary Data SD4.—Post-hoc analyses comparing body mass by sex among subspecies.

Supplementary Data D4. —Post-hoc analyses *(emmeans*) of GLMM with comparisons of body mass among subspecies (*C. h. natalensis*, *C. h. mahali*, and *C. h. pretoriae*) by sex.

Subspecies	Class	Estimate	T or z-value	P-value
C.h.n (325) vs. C.h.m (118)	All	0.01	0.44	1
C.h.n vs. *C.h.m*	F	-0.05	-1.5	1
C.h.n vs. *C.h.m*	M	0.08	2.2	0.42
C.h.p (156) vs. *C.h.m* (118)	All	-0.19	-6.7	< 0.001 **
C.h.p vs. *C.h.m*	F	-0.25	-6.1	< 0.001 **
C.h.p vs. *C.h.m*	M	-0.14	-3.5	0.008 *
C.h.p (156) vs. *C.h.n* (118)	All	-0.21	-8.7	< 0.001 **
C.h.p vs. *C.h.n*	F	-0.20	-5.8	< 0.001 **
C.h.p vs. *C.h.n*	M	0.04	1.3	1

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