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RESEARCH ARTICLE

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Genetic diversity and sex ratio of naked mole rat, *Heterocephalus glaber*, zoo populations

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The naked mole rat, *Heterocephalus glaber*, is a highly unusual mammal that displays a complex social system similar to that found in eusocial insects. Colonies of *H. glaber* are commonly maintained in zoo collections because they represent fascinating educational exhibits for the public. However, little is known about the genetic structure or sex ratio of captive populations of *H. glaber*. In this study, we developed a set of microsatellite markers to examine genetic variation in three captive zoo populations of *H. glaber*. We also studied sex ratio of these captive populations. Our goal was to determine levels of genetic variation within, and genetic differences between, captive populations of *H. glaber*. Overall, we found modest levels of genetic variation in zoo populations. We also uncovered little evidence for inbreeding within the captive populations. However, zoo populations did differ genetically, which may reflect the isolation of captive naked mole rat colonies. Finally, we found no evidence of biased sex ratios within colonies. Overall, our study documents levels of genetic variation and sex ratios in a captive eusocial mammalian population. Our results may provide insight into how to manage captive populations of *H. glaber*.

KEYWORDS
eusocial, genetic structure, genetic variation, molecular genetic analysis, sex ratio

1 | INTRODUCTION

Eusocial species live in highly-developed, interdependent societies (Wilson & Hölldobler, 2005). Eusocial insects, like ants and termites, are known for their division of labor in which individuals are tasked with specific jobs in the colony (Wilson, 1990). For example, some members of a colony reproduce while others aid in the care of the reproductive members and their offspring. Eusociality has been of considerable interest to evolutionary biologists because some individuals forgo personal reproduction in order to aid the reproduction of family members. The evolution of eusociality also involves the development of an array of cooperative behaviors that lead to colony success.

The evolution of eusociality can also affect sex ratio (Boomsma & Grafen, 1991; Bourke, 2015; Trivers & Hare, 1976). Sex allocation theory predicts that parents will adjust the sex ratios of their offspring according to their ability to invest in a specific sex and the resulting profit of that sex to the parent (Trivers & Willard, 1973). Therefore, sex ratios can be tied to the condition of the parents in species where the sexes have different future reproductive success. Interestingly, the genetic structure and interactions of eusocial species may affect the reproductive success of males and females of a colony and, therefore, may influence sex ratio evolution. Most investigations of sex ratios in eusocial species have focused on eusocial hymenopteran insects,

which are haplodiploid, (Boomsma & Grafen, 1991; Trivers & Hare, 1976). However, little is known about sex ratios in diploid, eusocial species.

The naked mole rat, *Heterocephalus glaber*, is one of very few known eusocial vertebrates (Jarvis, 1981). *H. glaber* is a unique mammal, known for its odd appearance, life history, and unusual social behaviors (Sherman, Jarvis, & Braude, 1992). Naked mole rats are native to Kenya, Ethiopia, Somalia, and Djibouti (Jarvis, 1981) and live within large subterranean colonies. These colonies are headed by a reproductive caste that is responsible for the production of new offspring. However, most colony functions, such as foraging and nest maintenance, are undertaken by a separate nonreproductive caste. Unlike eusocial Hymenoptera, males and females are found amongst the reproductive and nonreproductive castes. In addition, naked mole rat colonies are headed by a single queen that mates with multiple males. Both males and females remain in their natal colony, though some males do disperse (O' Riain, Jarvis, & Faulkes, 1996).

Previous studies have examined the population biology and genetics of wild *H. glaber* in Africa. One of the first studies to examine the genetic structure of *H. glaber* used DNA fingerprinting and uncovered high levels of genetic similarity and putative inbreeding within colonies. This study led to the widely accepted belief that inbreeding and low dispersal rates drove the evolution of eusociality in this species (Faulkes et al., 1997; Reeve, Westneat, Noon, Sherman, & Aquadro, 1990). However, more recent studies have uncovered evidence for outbreeding in wild populations (Braude, 2000; Ciszek, 2000; O' Riain et al., 1996). For example, Ingram et al. (2015), who used microsatellite markers to study a larger range of wild naked mole rats, found that levels of genetic diversity in *H. glaber* populations were higher than originally proposed.

The goal of this study was to understand the population structure and sex ratio of captive populations of *H. glaber* within zoos. We developed a set of polymorphic DNA microsatellite markers to genotype individuals from three zoos across the United States. We also used genetic and morphological analyses to determine the sex of individuals from these colonies. Overall, this investigation provides insight into the effect of captivity in shaping the genetic structure and sex ratio of eusocial animals. Our study also has implications for captive breeding programs of these unusual animals (Earnhardt, Thompson, & Marhevsky, 2001; Ivy & Lacy, 2012; Lacy, 2013).

2 | METHODS

2.1 | Sample genotyping

We assayed the multilocus genotype of a total of 89 *H. glaber* individuals sampled from three zoos including Zoo Atlanta (ZA, 2013–2014; $n = 60$), San Diego Zoo (SDZ, 2006–2013, $n = 11$), and the Smithsonian National Zoological Park (SNZP, 2015, $n = 18$). Individuals from Zoo Atlanta were part of a single colony, transferred from Houston Zoo, which received their colony from Point Defiance Zoo in Tacoma, Washington. The San Diego Zoo samples were derived from four in-house colonies which were originally initiated from colonies in the Philadelphia Zoo. The population from Smithsonian National Zoological Park also originated from the Philadelphia Zoo, with all but one individual coming from a single colony.

All individuals analyzed in this study died of natural causes within their colonies and were then frozen at −80 °C for subsequent analyses. Sampled individuals were non-reproductive members of the colony that varied in age. However, one individual from San Diego Zoo was noted to be a putative reproductive female who died of aggression. DNA was extracted from skin biopsies of individuals using a modified Chelex protocol (Goodisman, Matthews, & Crozier, 2001).

New microsatellite primers for *H. glaber* were developed by analyzing the *H. glaber* genome v1.1 (Appendix Table A1) (Kim et al., 2011). Microsatellite regions were located using the program MISA (Thiel, Michalek, Varshney, & Graner, 2003). Flanking primer regions were developed using Primer3 v 2.3.7 (Untergasser et al., 2012) with parameters set as follows: product size between 100 and 1,000 bp with the optimal size of 200 bp, primer size between 18 and 27 bp with an optimal length of 20 bp, annealing temperature between 57 °C and 63 °C with an optimal annealing temperature of 60 °C, and G—C content between 20% and 80%. Optimal annealing temperature for PCR primers were discerned using a gradient PCR machine. Further information on primers can be found in the NCBI Probe Database (Accession Pr032825906-Pr032825937). We also genotyped individuals with *H. glaber* primers originally developed by Ingram, Troendle, Gill, and Honeycutt (2014) (Hglab01, Hglab03, Hglab07, Hglab08, Hglab09, Hglab10, Hglab13, Hglab14, Hglab17, Hglab18, Hglab19, Hglab22).

Many loci were PCR-amplified with "traditional" fluorescently labeled primers as detailed in Hoffman, Kovacs, and Goodisman (2008). For traditional PCR, forward primers were labeled with one of the fluorescent dyes 6-FAM, 5-HEX, or TAMRA. PCRs were carried out in a final volume of 15 µl, with 1.0 µl of genomic DNA, 6.9 µl of sterile deionized water, 2.4 µl of 25 mM of $MgCl_2$, 1.5 µl 10× PCR buffer, 1.2 µl of 2.5 µM dNTPs, 0.75 µl of both forward and reverse primer at a concentration of 10 µM, and 0.5 µl of *Taq* DNA polymerase. PCR cycling profiles for traditional reactions started with a denaturation at 94 °C for 2 min, and then proceeded with 30 or 35 cycles of 93 °C for 30 s, primer-specific annealing temperature for 30 s, 72 °C extension for 30 s, all followed by a final extension of 72 °C for 10 min.

Although many loci were amplified using traditional PCR, some loci were amplified using a "non-traditional" M13-nested-PCR method (Schuelke, 2000) (Appendix Table A1). This M13-nested PCR method requires a universal sequence to be added to each forward primer. This universal sequence binds to a fluorescently labeled primer during the PCR. Non-traditional PCRs were carried out in a final volume of 15 µl, with 1.0 µl of genomic DNA, 6.71 µl of sterile deionized water, 2.4 µl of 25 mM $MgCl_2$, 1.5 µl of 10× PCR buffer, 1.2 µl of 2.5 mM dNTPs, 0.19 µl of 10 µM locus-specific forward primer, 0.75 µl of 10 µM fluorescently labeled universal forward primer, 0.75 µl of 10 µM reverse primer, and 0.5 µl of *Taq* polymerase. The PCR cycling profiles for the M13-nested PCR method began with an initial denaturation at 94 °C for 5 min, and then proceeded with 30 cycles of 94 °C for 30 s, the designated primer annealing temperature for 30 s, and 72 °C for

30 s, followed by eight cycles of 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 30 s. Finally, there was an extension of 72 °C for 10 min. All PCRs included negative controls to monitor for contamination and PCRs were generally not multiplexed.

Regardless of the PCR amplification method used, the resulting PCR amplicons were run on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Alleles were scored manually using GeneMapper (SoftGenetics). We genotyped all individuals at 44 microsatellite loci (Appendix Table A2). However, two loci (Hgla_2793 and Hgla_3591) were not readily scored and so were removed from the rest of the analyses. Thus, each naked mole rat was assayed at 42 microsatellite loci.

The sexes of individuals were determined genetically using a multiplex PCR assay (Katsushima et al., 2010). This protocol jointly amplifies part of the Y-linked *DBY* gene, which serves to identify the sex of the target individual, and the 16S rRNA gene, which acts as a PCR-amplification control. Therefore, the presence of two PCR products indicates the sample was derived from a male, while a single product appears if the sample was derived from a female. The sexes of individuals from San Diego Zoo were also determined by dissection, which acted as a control for the genetic sex ratio analyses. We used a χ^2 goodness-of-fit test to investigate if the sex ratios of the zoo populations deviated from 50:50.

2.2 | Genetic analyses

Genetic diversity measures, including number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{is}), were calculated with the program GenAlEx v 6.5 (Peakall & Smouse, 2012) for each zoo population. Kruskal–Wallis Tests were performed to see if there were significant differences in these genetic diversity measures across the three zoo populations. The probability test of GENEPOP v 4.6 was used to test for deviations of genotype frequencies from Hardy–Weinberg equilibrium and for the presence of linkage disequilibrium among loci (Rousset, 2008). Bonferroni corrections were used to adjust for multiple testing. The level of genetic differentiation between zoo populations was measured with Weirs and Cockerham's F_{st} using FSTAT v 2.9.3 (Goudet, 1995; Weir & Cockerham, 1984).

Individuals were grouped into putative populations, or genetic clusters, using the program STRUCTURE v 2.2 (Pritchard, Stephens, & Donnelly, 2000). The number of genetic clusters (*K*) present across all the zoos was identified using different simulations, each under the assumption of a different *K* value (1–4). To account for individuals with mixed ancestry, we used an admixture model with uncorrelated allele frequencies. We also utilized the LOCPRIOR model to use sampling location to inform clustering. For each *K*, we ran each simulation 10 times with 10,000 steps of burn-in and 100,000 MCMC iterations. The most likely value of *K* was identified based on log likelihood and the Δ*K* method developed by Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl & Vonholdt, 2012; Evanno, Regnaut, & Goudet, 2005; Pritchard et al., 2000). CLUMPP v 1.1.2 was then used to align the 10 replicate runs for each set of *K* simulations (Jakobsson & Rosenberg, 2007). The clustering results were visualized with Distruct v 1.1 (Rosenberg, 2004).

3 | RESULTS

3.1 | Genetic diversity

We examined the levels of genetic diversity of the ZA, SNZP, and SDZ *H. glaber* populations at 42 microsatellite loci. A total of 24 of those loci were monomorphic across all of populations (Table 1). Our analysis did not detect significant deviations from Hardy–Weinberg equilibrium in the SNZP ($\chi^2_{df=22} = 14.49$; $p = 0.883$; Kruskal–Wallis Test) or the SDZ ($\chi^2_{df=30} = 20.51$; $p = 0.902$) populations (Table 2). However, there was evidence for deviations from Hardy-Weinberg equilibrium in the ZA population ($\chi^2_{df=26} = 66.24$; $p < 0.001$). In contrast, we found no evidence for significant linkage disequilibrium between any pair of loci in any population.

We compared the levels of genetic diversity between the zoo populations (Table 1). We found no significant differences in number of alleles ($\chi^2_{df=3} = 3.61$, $p = 0.307$; Kruskal–Wallis Test), number of effective alleles ($\chi^2_{df=40} = 41.58$, $p = 0.402$), observed heterozygosity ($\chi^2_{df=36} = 40.62$, $p = 0.274$), or expected heterozygosity ($\chi^2_{df=38} = 36.62$, $p = 0.533$) among populations. Each population had a few private alleles: one in ZA, four in SNZP, and seven in SDZ.

Levels of F_{is}, which measures the degree of inbreeding in a population, are presented in Table 1. Negative values of F_{is} indicate that there is an excess of heterozygosity relative to expectations, whereas positive values of F_{is} indicate a deficit of heterozygosity relative to expectations. We found a significant, negative value of F_{is} for the ZA population. However, F_{is} levels were not significantly different from zero for both the SNZP and SDZ populations.

3.2 | Genetic differentiation between colonies

We performed allelic probability tests for each population pair across all loci using the program GENEPOP to determine if allele frequencies differed among the zoo populations. Each of the three pairwise comparisons between zoos was highly significant ($p < 0.001$). We also found that F_{ST} estimates were rather high, with pairwise values between ZA and SNZP, ZA and SDZ, and SDZ and SNZP equal to 0.498, 0.376, and 0.446, respectively. Therefore, overall, we found high and significant genetic differentiation between all zoo populations.

Analysis of the relationships among the zoo populations using the program STRUCTURE revealed the relationships among the three zoo populations (Figure 1). Our analyses suggested that the three zoo populations actually represented two distinct clusters ($K = 2$; $\Delta K = 5875.20$), rather than the expected three clusters ($K = 3$, $\Delta K = 159.99$) (Figure 1a). Cluster 1 was composed of all individuals from the ZA population and Cluster 2 consisted of all individuals from the SNZP population. Surprisingly, there was some evidence of admixture between the two clusters in the SDZ population. Interestingly, however, when we set the number of populations to

TABLE 1 Genetic diversity measures of *H. glaber* from Zoo Atlanta, Smithsonian National Zoological Park, and San Diego Zoo for genetically variable microsatellite loci

	Zoo Atlanta					Smithsonian National Zoological Park					San Diego Zoo				
Microsatellite locus	N_a	N_e	H_o	H_e	F_{is}	N_a	N_e	H_o	H_e	F_{is}	N_a	N_e	H_o	H_e	F_{is}
Hgla_6757.2	2	1.806	0.569	0.446	−0.275	1	1.000	0.000	0.000	–	2	1.385	0.333	0.278	−0.200
Hgla_7804	2	1.636	0.528	0.389	−0.359	2	1.895	0.529	0.472	−0.121	2	1.984	0.364	0.496	0.267
Hgla_7221.2	1	1.000	0.000	0.000	–	1	1.000	0.000	0.000	–	2	1.980	0.700	0.495	−0.414
Hgla_2663	1	1.000	0.000	0.000	–	2	1.882	0.625	0.469	−0.333	2	1.342	0.300	0.255	−0.176
Hgla_4233.1	2	1.718	0.262	0.418	0.374	2	2.000	0.111	0.500	0.778	2	1.471	0.000	0.320	1.000
Hgla_6197	2	1.160	0.149	0.138	−0.080	2	1.220	0.200	0.180	−0.111	1	1.000	0.000	0.000	–
Hglab17	1	1.000	0.000	0.000	–	2	2.000	0.385	0.500	0.231	2	2.000	0.500	0.500	0.000
Hgla_9415	2	1.518	0.436	0.341	−0.279	2	1.205	0.188	0.170	−0.103	2	1.976	0.667	0.494	−0.350
Hglab03	2	1.552	0.463	0.356	−0.301	3	1.947	0.625	0.486	−0.285	2	1.753	0.375	0.430	0.127
Hglab07	1	1.000	0.000	0.000	–	2	1.064	0.063	0.061	−0.032	3	2.800	0.429	0.643	0.333
Hglab08	1	1.000	0.000	0.000	–	2	1.080	0.077	0.074	−0.040	2	1.280	0.250	0.219	−0.143
Hglab14	2	1.494	0.418	0.331	−0.264	2	1.074	0.071	0.069	−0.037	2	1.960	0.571	0.490	−0.167
Hglab18	2	1.625	0.519	0.384	−0.351	1	1.000	0.000	0.000	–	2	1.508	0.429	0.337	−0.273
Hglab09	2	1.766	0.600	0.434	−0.383	3	1.471	0.313	0.320	0.024	3	2.418	0.667	0.586	−0.137
Hglab10	2	1.791	0.415	0.442	0.061	2	1.936	0.455	0.483	0.060	1	1.000	0.000	0.000	–
Hglab13	3	2.062	0.550	0.515	−0.068	2	1.600	0.500	0.375	−0.333	3	1.815	0.286	0.449	0.364
Hglab19	2	1.999	0.380	0.500	0.240	1	1.000	0.000	0.000	–	3	2.323	0.500	0.569	0.122
Hglab22	2	1.975	0.472	0.494	0.044	2	1.912	0.357	0.477	0.251	4	3.459	0.500	0.711	0.297
MEAN	1.333	1.217	0.137	0.124	−0.126	1.381	1.197	0.107	0.110	−0.004	1.524	1.368	0.164	0.173	0.041
S.E.	0.081	0.055	0.034	0.030	0.038	0.090	0.055	0.030	0.029	0.044	0.119	0.091	0.037	0.037	0.055

F_{is}, inbreeding coefficient; H_e, expected heterozygosity; H_o, observed heterozygosity; N_a, observed number of alleles; N_e, effective number of alleles.

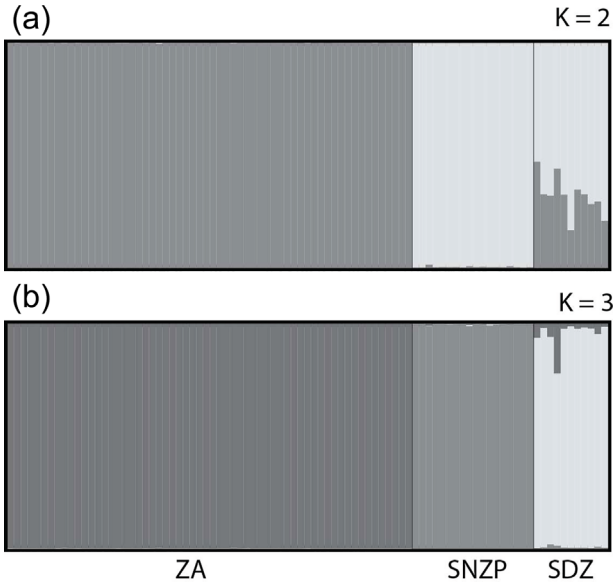

FIGURE 1 Estimated membership coefficients for *H. glaber* individuals from Zoo Atlanta (ZA), Smithsonian National Zoological Park (SNZP), and San Diego Zoo (SDZ) colonies. *K* represents the number of putative populations into which individuals are clustered. Each line represents an individual, the shade of which corresponds to the estimated membership of that individual in a certain cluster. (a) *K* = 2. (b) *K* = 3

K = 3, we recovered three clusters that corresponded to the three zoo populations (Figure 1b).

3.3 | Genetic sex identification

We determined the sex of many of the sampled naked mole rats (Katsushima et al., 2010) (Appendix Table A2). Individuals from SDZ were sexed both genetically and through dissections performed at the zoo. The sex identified through dissections matched the sex resulting from the genetic analyses for all individuals, thus validating the genetic method of sex determination. The ZA population consisted of 26 females and 34 males, and the sex ratio did not differ significantly from equality ($\chi^2_{df=1} = 1.07$; $p = 0.302$; χ^2 goodness-of-fit test). The sex ratios (f:m) of the SNZP and SDZ samples were 5:5 ($\chi^2_{df=1} = 0$; $p = 1$) and 6:4, respectively ($\chi^2_{df=1} = 0.4$; $p = 0.527$). Thus there was no evidence that sex ratio differed from equality in any of the captive populations.

4 | DISCUSSION

The goal of this study was to investigate the levels of genetic variation and sex ratios of captive colonies of naked mole rats. We sampled individuals from colonies at Zoo Atlanta (Atlanta, GA; ZA), the San Diego Zoo (San Diego, CA; SDZ), and the Smithsonian National Zoological Park (Washington, DC; SNZP). We found that levels of genetic variation in our captive populations were modest (Table 1). Indeed, the general metrics of variability were lower than those for most of the native, wild populations of naked mole rats analyzed by Ingram et al. (2015). However, levels of heterozygosity were similar to those in wild populations sampled central and south of the Tana River (Ingram et al., 2015). Thus, the captive zoo populations analyzed in our study seem to have levels of variation similar to those of at least some wild populations of this species.

We detected deviations from Hardy–Weinberg equilibrium in one (ZA) of our three populations. The estimate of F_{is} for ZA was significant and negative. Therefore, there was an excess of heterozygotes in the ZA population relative to expectations. Such deviations are actually expected in this case and likely arise because colonies represent families of related individuals. Therefore, sampled individuals are not genetically independent as one would expect in a randomly mating population.

Early genetic studies of natural populations of *H. glaber* suggested a high level of inbreeding for naked mole rats, which was believed to have facilitated the evolution of eusociality (Reeve et al., 1990). However, more recent studies suggest that the previously observed high level of inbreeding was an artifact of sampling bias. In particular, Ingram et al. (2015) investigated genetic structure of previously-unstudied naked mole rat populations (Faulkes et al., 1997; Reeve et al., 1990). They found that inbreeding coefficients for their populations varied substantially and were sometimes strongly positive and sometimes strongly negative. Specifically, they confirmed that the previously studied naked mole rat populations had positive inbreeding coefficients. However, these high levels of inbreeding were likely linked to a founder effect associated with geographic isolation. In contrast, some of the newly analyzed populations displayed negative inbreeding coefficients (Ingram et al., 2015). Therefore, natural *H. glaber* colonies are apparently less inbred than previously thought.

Zoo populations of naked mole rats are clearly isolated from one another. So one might expect to find a high level of inbreeding within zoo colonies. When we compared the inbreeding coefficients of our zoo samples to those from wild populations (Ingram et al., 2015), we found that the magnitudes of the inbreeding coefficient (F_{is}) from the zoo populations were similar to those from wild populations sampled north of the Tana River. However, comparisons of the levels of inbreeding between captive and wild populations of naked mole rats must be viewed with caution because of the extremely different conditions under which these populations live. The natural population estimates were obtained by sampling across multiple colonies and therefore provide information on population-level attributes. In contrast, the estimates obtained from the captive zoo samples in this study were derived from single colonies and therefore provide information about genetic relationships within families.

We detected population structure among the naked mole rat zoo populations indicating that the zoo populations differed genetically. When we grouped individuals based off their multilocus genotype, we found that all zoo samples could be clustered into two groups. Individuals from ZA made up one cluster, individuals from SNZP made up the second cluster, and individuals from SDZ were admixed (Figure 1). Therefore, individuals from ZA and SNZP were most

divergent. However, when we assumed that there were three clusters, all three populations could be differentiated.

The genetic structure between the zoo populations likely arose, in part, through founder effects associated with the creation of new colonies. Currently, there are few records tracking the specific sources and transfer of individual naked mole rats among zoos. In fact, many naked mole rat colonies in U.S. zoos have been sourced by a private collector (pers. comm.). The records available show that ZA's naked mole rat colony originates from Point Defiance Zoo (Tacoma, WA) by way of Houston Zoo (Houston, TX). SNZP received their colony from Philadelphia Zoo in 1991. SDZ also received some samples from Philadelphia Zoo in 1992. Despite the fact that both SNZP and SDZ colonies originated from the Philadelphia Zoo, the SDZ and ZA colonies were actually more similar to each other. This similarity may reflect the maintenance of some ancestral variation between colonies and, perhaps, the influence of genetic drift and small sample size in this study. Regardless, it is notable that we were able to distinguish the three zoo colonies using genetic techniques (Figure 1b). Thus, increased sampling and genetic analyses may allow for further reconstruction of the relationships of captive populations of this species and ultimate identification of putative natural source populations.

Because zoo colonies are spatially fragmented with strongly restricted migration, one would expect to see genetic differentiation of populations increase through genetic drift over time (Frankham, 2008). Indeed, genetic drift is likely to have a very strong effect in captive naked mole rats, because only a single female breeds at any one time. However, extensive differentiation due to genetic drift may not have occurred among our sampled populations as yet, because these colonies have not been in captivity for very long. For example, the zoo population have only been in captivity for ~25 years. Future studies incorporating the results presented in this investigation may be able to determine if drift does have a strong effect on allele frequencies among captive naked mole rat colonies.

Our genetic results may have implications for management strategies for *H. glaber* captive populations. Naked mole rats in Association of Zoos & Aquariums (AZA) institutions are not being managed under a Species Survival Plan because the species is not endangered. Moreover, there seems to be little evidence that captive populations are suffering low fitness. However, given their peculiar breeding system, and the evident propensity for long-term genetic concerns in these isolated populations, we recommend that a cooperative managed breeding plan, with regular genetic screenings, be implemented to ensure the long-term viability of the species across AZA institutions. Ideally, management of this species in captive populations would include tracking of the origin and fate of individual mole rats from new colonies. Potential losses in heterozygosity within zoo colonies over the long term may warrant the implementation of more complex programs including the incorporation of individuals from multiple distinct colonies into breeding programs. However, such practices may not be necessary at this time since the captive zoo populations seem to show reasonable levels of genetic variation and viability.

We examined the sex ratio of each zoo population. We note that it is possible that our opportunistic sampling scheme may have biased our results in some way. For example, if individuals of a particular sex were more likely to die as pups, then our results may be biased, because almost all of our samples were deceased pups. Moreover, our sample sizes from the SDZ and the SNZP were quite small and therefore provided little power to detect significant sex ratio deviations. However, the sample size from ZA was reasonable. Thus, our investigation provides a first approximation of sex ratio in naked mole rats.

Overall, our analyses did not reveal any evidence for sex-ratio bias within captive naked mole rat colonies. This result is in accordance with data from other mole rat species, which suggest that the cost to produce each sex is equal (Begall & Burda, 1998; Bennett & Faulkes, 2000; Fisher, 1930). However, there is some evidence for a male-biased sex ratio in the offspring of wild-captured giant mole-rats (*Fukomys mechowii*), though this bias is not found amongst adults (Kawalika & Burda, 2007). Interestingly, captive colonies of the giant mole-rat have a high female-biased neonate sex-ratio (Kawalika & Burda, 2007; Scharff et al., 1999). It will be important to determine if captive and wild populations of *H. glaber* also show differences in sex ratio.

Most research conducted on sex ratios in eusocial species has focused on haplodiploid eusocial insects (Boomsma & Grafen, 1991; Queller & Strassmann, 1998; Trivers & Hare, 1976). Haplodiploidy leads to an asymmetry in the level of relatedness between the sexes such that workers are predicted to invest more resources into the raising of sisters than brothers, which may lead to female-biased sex investment ratio (Trivers & Hare, 1976). Naked mole rats are diploid so there is no relatedness asymmetry. Therefore, one might predict a lack of sex ratio bias in eusocial diploids. However, there are other mechanisms that could lead to adaptive sex ratio bias in diploid species, such as local resource competition (Silk, 1983), local resource enhancement, or helper repayment (Emlen, Emlen, & Levin, 1986; Gowaty & Lennartz, 1985). Investigations in the captive Damaraland mole-rat (*Fukomys damarensis*), another putatively eusocial rodent, partially support each of these three mechanisms (Lutermann, Cameron, Raihani, & Bennett, 2014). Thus, factors such as sex-biased dispersal, differences in helping between the sexes, and the condition of the reproductives may play a role in shaping the sex ratio of a colony. More theoretical work and extensive sampling must be conducted to further understand the association between eusociality and sex ratios in naked mole rats in both captive and wild populations.

5 | CONCLUSIONS

1. Naked mole rats are fascinating animals that display complex social systems similar to those found in eusocial insects.
2. We used genetic techniques to examine levels of genetic variation and sex ratios in three zoo populations of naked mole rats.
3. We found that zoo populations displayed modest levels of genetic variation, substantial genetic differences among populations, and relatively little inbreeding.

4. We found no evidence for sex ratio bias within zoo populations.
5. This research provides insight on the levels of genetic variation and sex ratio of captive naked mole rats, which may aid in the management and care of these remarkable mammals.

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APPENDIX

TABLE A1 New microsatellite loci developed for *H. glaber*

Locus	Sequence (5′–3′)	Repeat	Size (bp)	T_a	PCR Method	Label
Hgla_243.1	F: CTACTGAGCTGCTTCGAGCC R: TGCAGAAGTCATCCTTGGCA	$(AC)_9$	249	69	Traditional	6-FAM
Hgla_330	F: AGGGTTTTCCCAGTCACGACGTTACCTGTCTGTGTGCATGTGT R: CAAGCACACACCTGGAGCTA	$(TG)_8$	234	68	m13	
Hgla_857	F: AGGGTTTTCCCAGTCACGACGTTTGTCTTGGTGCCCACTTACC R: TCACATGATGGCAACTGGCT	$(GA)_6$	252	68	m13	
Hgla_2663[a]	F: AGGGTTTTCCCAGTCACGACGTTCCCACTCCATCTCTCAAGGC R: TGCCTGTAATCCCAACAGCT	$(TTGT)_7$	263	68	m13	
Hgla_2681	F: CCCATGATCACAGCGAGACA R: AGTTTGCCCTCCAGTTTCCT	$(AATA)_5$	254	68	Traditional	5HEX
Hgla_2793[b]	F: AGGGTTTTCCCAGTCACGACGTTACAGAGAGAGGGAGAGAAAGAGA R: TGTGTGCTGAAGATGACATCCA	$(AG)_{14}$	220	67	m13	
Hgla_3223	F: AGGGTTTTCCCAGTCACGACGTTCCCCACCTACCCACCTATGA R: TGGATTCTGGTGTGGGTTCA	$(AT)_8$	196	66	m13	
Hgla_3322	F: AGGGTTTTCCCAGTCACGACGTTTGTTCTAACACAGTTAAGTTGACTTCA R: ACACAGATTCACAAAACTGTTAGCA	$(TA)_{14}$	280	67	m13	
Hgla_3591[b]	F: AGGGTTTTCCCAGTCACGACGTTTCACTGACTGCAACCATAGGT R: TGCTAATGTTTAACAACTAGCTTTCCA	$(AT)_{16}$	254	67	m13	
Hgla_4233.1[a]	F: AGGGTTTTCCCAGTCACGACGTTAGCCGCCAACTGTGAACTAA R: AGTAAGTACCATTTGACAAAAAGCT	$(AC)_{11}$	236	66	m13	
Hgla_4598	F: AGGGTTTTCCCAGTCACGACGTTATGACACAATGCAGGGGAGG R: AGGCAGTGGCACAAGATGAA	$(GT)_6$	231	67	m13	
Hgla_4642	F: AGGGTTTTCCCAGTCACGACGTTGCGGGGCATTTGTTTCCTTT R: AACTCAGGACCTCGTGCTTG	$(TG)_7$	231	71	m13	
Hgla_6197[a]	F: AGGGTTTTCCCAGTCACGACGTTGCGGACCCTAAATCTGGCTT R: ACACCATGCTCACACACACA	$(TG)_{13}$	276	68	m13	
Hgla_6226	F: AGGGTTTTCCCAGTCACGACGTTAAATGCAGTGTTTGGCAGGG R: GCACCCACTGCTTGTCTGTA	$(AC)_8$	264	71	m13	
Hgla_6655	F: AGGGTTTTCCCAGTCACGACGTTTCTGTGCACGTACCAACTCC R: TGTGGACCCTGATGCATGAC	$(GT)_6$	240	71	m13	
Hgla_6757.2[a]	F: AGGGTTTTCCCAGTCACGACGTTAATCTCTCTCCCCCAGCTGT R: TATTGGATGACACCCGGCAG	$(AC)_{15}$	168	67	m13	
Hgla_7076	F: GGCTTGGCCTGAACTGTGTA R: TCAGTGAGCATCTTGTACAAGTGA	$(GT)_7$	157	66	Traditional	5HEX
Hgla_7146	F: AGGGTTTTCCCAGTCACGACGTTGGCGGGAGTAATGGACACAG R: CAACATGCCTGGCTGGAAAC	$(CT)_7$	215	69	m13	
Hgla_7221.2[a]	F: TCAACTGTCTGGGATCCCCT R: CTGTGGCCCTTGGAACAGTA	$(CA)_{13}$	209	66	Traditional	TAMRA
Hgla_7269	F: CCCAGAGGACACACTGAAAGA R: CCACCTGTCTCAGCCTCCTA	$(TA)_6$	243	68	Traditional	TAMRA
Hgla_7285	F: AGGGTTTTCCCAGTCACGACGTTGCTTTGCTCTTGTTGCCCAA R: GCTCAGTGGTTCTGCTGAGT	$(TG)_7$	205	67	m13	
Hgla_7633	F: AGGGTTTTCCCAGTCACGACGTTAAGTGAGAACATACACCCATGT R: GACCGGGAGAGCTAGAATGC	$(TC)_9$	195	71	m13	
Hgla_7797.1	F: AAGTGAGAACATACACCCATGT R: GACCGGGAGAGCTAGAATGC	$(GT)_6$	144	68	Traditional	TAMRA
Hgla_7797.2	F: GCATTCTAGCTCTCCCGGTC R: TTCTGGAGGGATAGGTGGCA	$(TC)_6$	277	66	Traditional	6-FAM

(Continues)

TABLE A1 (Continued)

Locus	Sequence (5′–3′)	Repeat	Size (bp)	T_a	PCR Method	Label
Hgla_7804[a]	F: CGTGTCCTCTTGGTGTGACA R: ACAGTCTGCCTTCACGATCG	$(AC)_{14}$	110	66	Traditional	TAMRA
Hgla_7996	F: TCACAAGCACAAGGTCCCAG R: CTCCTCCCTTGATCCCTCCA	$(AATA)_5$	200	66	Traditional	6-FAM
Hgla_8448.2	F: GGGCTTCTTCACCCAACAGT R: GCCAGCCTGAGATCCTGTTT	$(ATTT)_5$	198	66	Traditional	5HEX
Hgla_9217	F: ACTGTGACGTGATAAAGTGGCT R: CAGTAGCAGAGCCTGAGCAT	$(TA)_8$	181	68	Traditional	TAMRA
Hgla_9338	F: AGGGTTTTCCCAGTCACGACGTTTCTGTGGTCTTTCTCACACAC R: TGACAAAGTTGGACTATGCACA	$(AC)_7$	217	67	m13	
Hgla_9415[a]	F: AGGGTTTTCCCAGTCACGACGTTTGCCGAGAAGGTGCAGAAAT R: GCCTGGGCAAACTAGTGAGA	$(TC)_8$	258	69	m13	
Hgla_10012	F: AGGGTTTTCCCAGTCACGACGTTGATTTCTAGTGTGCACGCGC R: GCAAGTTCAAGCCCACCATG	$(TG)_7$	146	71	m13	
Hgla_10193	F: AGTGATAAGGGGCTGGGGAT R: GTTCAAGCCCAAGCCACATG	$(AC)_7$	181	68	Traditional	TAMRA

T_a optimized annealing temperature.
PCR method is the PCR protocol used for the specified set of primers; see main text for details.
[a]Polymorphic loci.
[b]Unscorable loci.

TABLE A2 Collection dates and sexes for all analyzed *H. glaber* individuals

Zoo	Collection date	Sex
Zoo Atlanta, Atlanta, GA	May 29, 2013	M
	May 29, 2013	M
	May 29, 2013	F
	May 29, 2013	F
	May 29, 2013	F
	May 29, 2013	M
	January 25, 2014	F
	January 25, 2014	F
	January 25, 2014	F
	January 25, 2014	F
	January 25, 2014	M
	January 25, 2014	F
	January 25, 2014	M
	January 25, 2014	M
	January 25, 2014	M
	January 25, 2014	F
	January 25, 2014	F
	January 25, 2014	M
	January 25, 2014	M
	January 25, 2014	M
	January 25, 2014	M

(Continues)

TABLE A2 (Continued)

Zoo	Collection date	Sex
	January 25, 2014	M
	January 25, 2014	F
	January 25, 2014	M
	January 25, 2014	M
	January 25, 2014	F
	April 17, 2014	M
	April 17, 2014	F
	April 17, 2014	M
	April 17, 2014	M
	April 17, 2014	M
	April 17, 2014	F
	April 17, 2014	M
	April 17, 2014	F
	April 17, 2014	M
	April 17, 2014	M
	April 17, 2014	F
	April 17, 2014	M
	April 17, 2014	F
	April 17, 2014	M
	August 26, 2014	M
	August 26, 2014	M
	August 26, 2014	F
	August 26, 2014	F
	August 26, 2014	F
	August 26, 2014	F
	August 26, 2014	F
	August 26, 2014	M
	July 11, 2014	M
	July 11, 2014	M
	July 11, 2014	M
	July 11, 2014	M
	July 11, 2014	F
	July 11, 2014	M
	July 11, 2014	M
	July 11, 2014	M
	July 11, 2014	F
	July 11, 2014	F
	July 11, 2014	F
	July 11, 2014	M
Smithsonian National Zoological Park, Washington, DC	May 24, 2015	M
	May 24, 2015	F
	May 24, 2015	M
	May 24, 2015	M
	May 24, 2015	F

(Continues)

TABLE A2 (Continued)

Zoo	Collection date	Sex
	May 24, 2015	M
	Unknown	M
	Unknown	M
	Unknown	M
	Unknown	F
	Unknown	F
	November 20, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
	November 4, 2015	Unknown
San Diego Zoo, San Diego, CA	May 2, 2012	F
	April 7, 2013	F
	December 27, 2013	F
	May 3, 2012	F
	December 27, 2013	F
	November 7, 2010	F
	December 20, 2012	F
	November 7, 2010	M
	April 7, 2013	M
	April 7, 2013	M
	April 7, 2013	M