

## Cultural transmission of vocal dialect in the naked mole-rat

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**Abstract:** Naked mole-rats form some of the most cooperative groups in the animal kingdom, living in multi-generational colonies under the control of a single breeding queen. Yet, how they maintain this remarkable social organization is unknown. Here we show that the most common naked mole-rat vocalization, the soft chirp, is used to transmit information about group membership, creating distinct colony dialects. Audio playback experiments demonstrate individuals make preferential vocal responses to home colony dialects. Pups fostered into foreign colonies in early postnatal life learn the vocal dialect of their adoptive colonies suggesting vertical transmission and flexibility of vocal signatures. Dialect integrity is in part controlled by the queen, as loss of a queen decreases dialect cohesiveness which reemerges only with the ascendance of a new queen.

**One Sentence Summary:** Vocal culture in naked mole-rats supports colony identity and cohesion.

**Main Text:** The naked mole-rat (*Heterocephalus glaber*) was the first identified eusocial mammal (1) and has received much attention for an array of extreme physiological traits (2–4). Yet often overlooked is their constant peeping, chirruping, and grunting (5, 6) (Audio S1-S2). Complex patterns of acoustic communication exist throughout the animal kingdom (7) and decades of study: notably in songbirds (8), bats (9), cetaceans (10), and primates (11) have generated debate about the etiology of human language with compelling evidence for anatomical (12) genetic (13) and

cultural (14) drivers. The highly cooperative nature of naked mole-rat societies led us to investigate whether their vocalizations support social complexity.

The vocal repertoire of the naked mole-rat consists of 17-25 distinct vocalizations (6, 15). The most common vocalization, the soft chirp, serves as a greeting call previously shown to occur in a stereotyped call and response, i.e. antiphonal manner (16).

We recorded 36,190 soft chirps from 166 animals (7 colonies), housed in Berlin, Germany or Pretoria, South Africa over a period of two years. We developed an algorithm to automatically segment, trace and extract acoustic features of individual soft chirps (Fig. 1A). In developing our analysis pipeline, we included established parameters for vocalization analysis (17) and whenever possible spectrogram-extracted features which minimized variable background noise from recordings made across different locations and days. Using a type of supervised machine learning, the Random Forest Classifier (18) we analyzed eight soft chirp features, three from the soundwave (pitch, wiener entropy, and zero-crossings rate) and five from the soft chirp spectrogram (asymmetry, peak frequency, height, duration and slope) (Fig. 1A, fig.S1). Training the classifier with soft chirps from individual mole-rats we found it could reliably predict the identity of individuals within a colony (Fig. 1B, fig.S2).

Within naked mole-rat colonies reproductive suppression of nearly all colony members is necessary to sustain the colony with limited food resources and leads to strong xenophobia (19). As such, multiple mechanisms for maintaining the social integrity of the colony and for detecting intruders might be necessary. We next tested for colony-specific signatures (16) using soft chirps recorded from three colonies in Berlin (Colonies, B, M and T) and a fourth colony which has always been located in South Africa, (Colony D). Again, using a Random Forest classifier, we found that soft chirp features were highly predictive of colony identity (Fig. 2A,B fig. S3- S5) with asymmetry and peak frequency found to be the best spectrogram-derived features for colony separation (Fig.2C,D, 16). While we did not find rank, age or sex to be strongly predicted by soft chirp features (fig. S6), we observed a positive correlation with body size and soft chirp pitch (Supplementary Text, fig. S7).

We next tested if animals recognize information communicated via soft chirps. To test this, we employed a place preference assay in which individual animals were given access to two interconnected chambers. (Fig.3A top, Video S1), each equipped for simultaneous audio playback

and recording. Animals preferred to spend most time in the chamber with sound presentation regardless of which colony soft chirp playback (home or foreign) was played (Fig.3A). Animals frequently vocalized in response to the audio playback stimulus with their own soft chirp, consistent with the antiphonal behavior previously described (Fig. 3B) (16, 20). We observed very high responses rates when animals were presented with home colony audio playbacks, much higher and significantly different compared to responses to foreign colony playbacks (Fig. 3C).

Naked mole-rats might recognize individual voices from home colonies rather than colony dialects. To test this, we designed artificial stimuli, using two features: asymmetry and peak frequency (16). Artificial stimuli were designed such that our colony classifier categorized these vocalizations as “mock colony members” but did not overlap with any known individual in the colony (fig.S8). Remarkably, response rates were again higher for the mock home stimulus suggesting that naked mole-rats can distinguish colony specific features in vocalizations (Fig.3D,E, fig.S8). To test if peak frequency or asymmetry alone were sufficient for behavioral preference, we used a pure tone of 4.5 kHz (mean colony peak frequency) and a frequency-doubled stimulus (9.0 kHz with mean colony asymmetry). We observed responses to the pure tone alone, but virtually none to the frequency-doubled stimulus (Fig.3E, fig.S8). The preferential response to home colony dialect was still found in the presence of a conflicting olfactory cue in the test chamber (Fig. 3F).

If naked mole-rats use distinct colony dialects to differentiate themselves from neighboring colonies or as a mechanism for ensuring conformity within the colony, such dialects must be maintained across generations. We cross-fostered three individuals between colonies, a non-trivial task as queens are rare breeders that cannot be synchronized across colonies. An abandoned pup (pup Mi) was cross fostered from Colony T to Colony M (Fig.4A,D) and we simultaneously tracked two surviving foster-siblings born in Colony M (pups Ob and Ny, Fig. 4, B, C). In a second experiment, two orphaned pups (pups Da and Jo, Colony S) were fostered into two different colonies (Colony M and Colony T, respectively) (Fig.4E-G). We observed that adult vocalizations fully develop by about ~ 3 months (Supplementary Text, fig. S9, S10), so we examined pup dialects at time points later than 6 months post-fostering. We tested foster pup vocalizations on our colony classifier, which classified the pups as belonging to one of five test colonies (including birth and foster colonies). In all three successful foster experiments, the new colony dialect was adopted with correct prediction rates between 59-95% (Fig. 4H).

Finally, we investigated if the queen's presence might influence the vocal signature of the colony (Supplementary Text). During the course of this study Colony S consecutively lost two queens (Fig. 4I, fig. S11) allowing us to record soft chirps during queen epochs and subsequent periods of anarchy. Individual variability of several features including peak frequency was higher during periods of anarchy (Fig. 4J, fig. S12) and classification accuracy of the colony dialect decreased during periods of anarchy (Fig. 4K, fig. S12), suggesting the presence of the queen enhances dialect cohesiveness.

Acoustic communication of social information has been observed in multiple mammalian species: bats (9), primates (11), cetaceans (10), pachyderms (21), and carnivores (22) and here we expand this group to include a member of the order Rodentia. More work is needed to resolve if naked mole-rats are capable of true production learning as exemplified in songbirds or if like many non-human primates they are exceptionally good usage learners (23). With a simple vocal greeting, humans convey individual identity (unique voice) and cultural identity (dialect usage), and here we show that naked mole-rats also signal social membership with dialect usage. Dialect features can be transmitted across generations a remarkable and hitherto undescribed feat for a rodent species, supporting an accumulating body of evidence that social complexity evolved hand in hand with vocal complexity.

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**Acknowledgments:** We thank J. Reznick, U. Koch, A. Rossi, and O. Eigenbrod for helpful comments on the manuscript, G. Pflanz, N. Schrim, and A. Mühlenberg for naked mole-rat husbandry, R. Hodge for illustrations and C.O. Lewin for Supplementary Movies. **Funding:** This work was supported by grants from the European Research Council (Advanced Grant 294678 to G.R.L) and a South African Research Chair for Mammalian Behavioral Research to N.C.B. We thank O. Daumke for additional salary funding. **Author contributions:** This project was conceived by A.J.B. and G.R.L. who together wrote the manuscript with input from all authors. Behavioral experiments were performed by A.J.B., N.C.B., D.W.H., and L.M. Machine learning and data analysis pipelines were developed by G.V with input from A.J.B. **Competing interests:**

The authors declare no competing interests. **Data and materials availability:** All data is available in the manuscript or the Supplementary Materials.

**Supplementary Materials:**

Materials and Methods

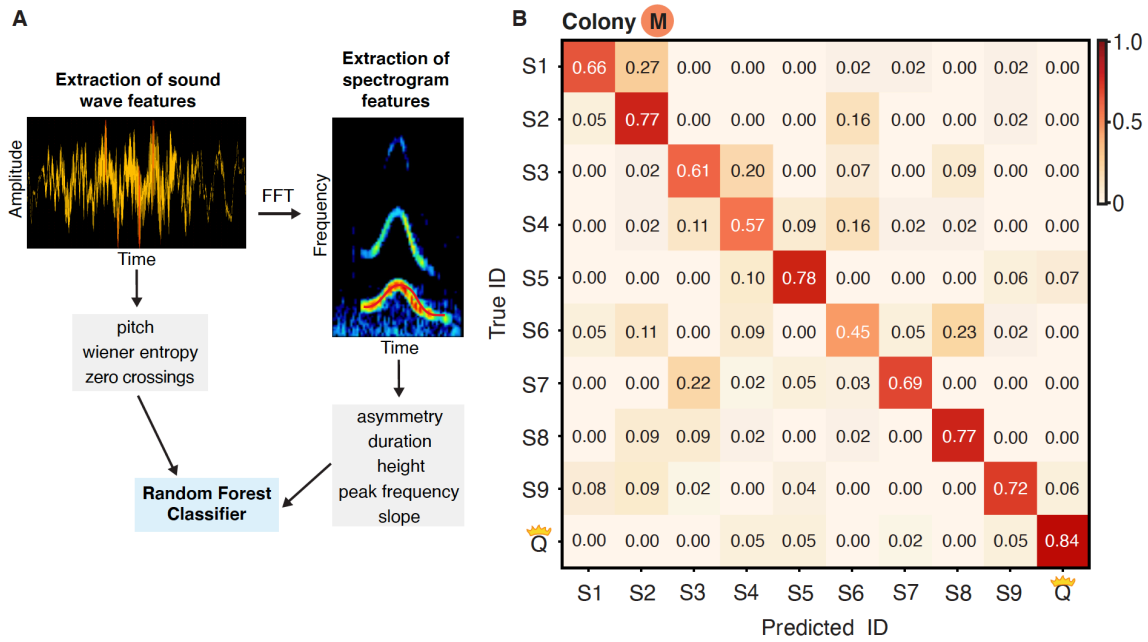
Supplementary Text

Figures S1-S12

References (24-30)

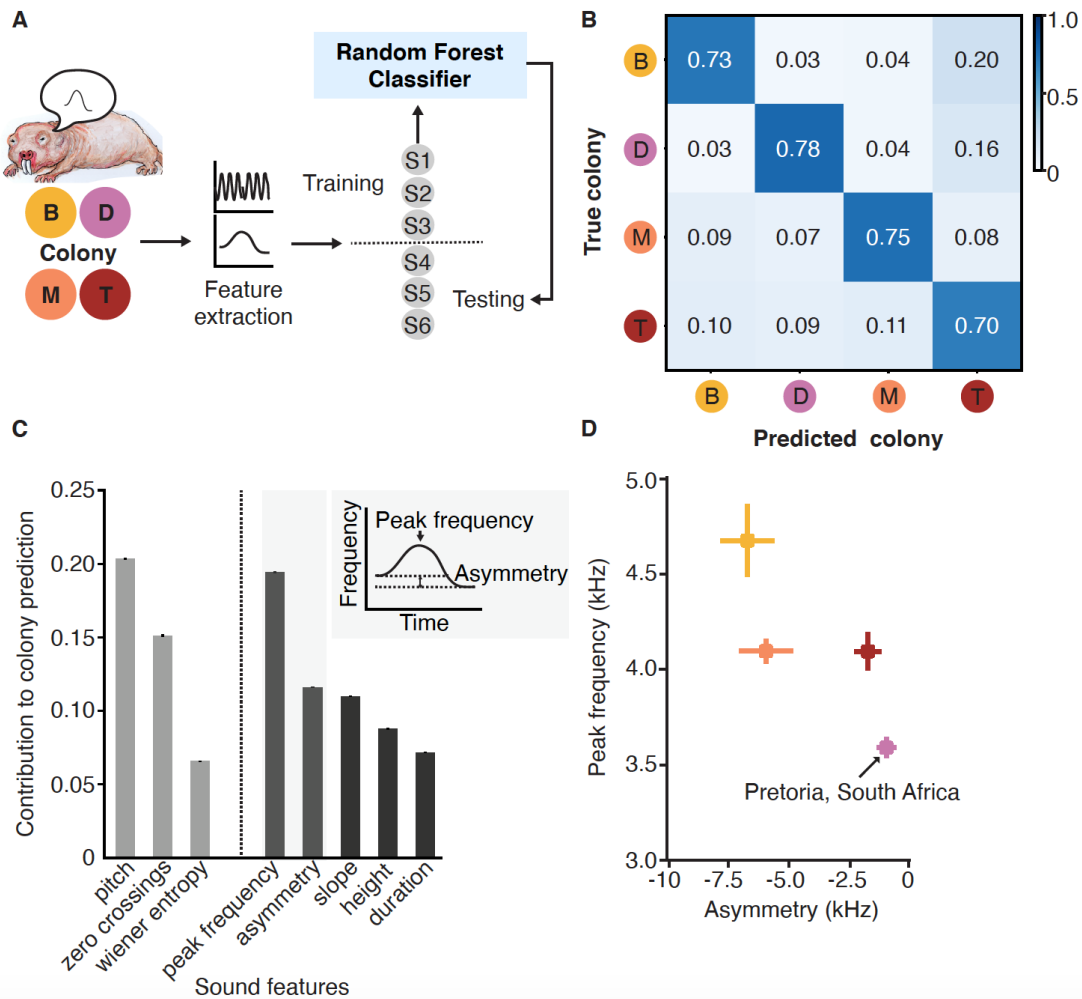
Movies S1-S2

Audio Files S1-S2

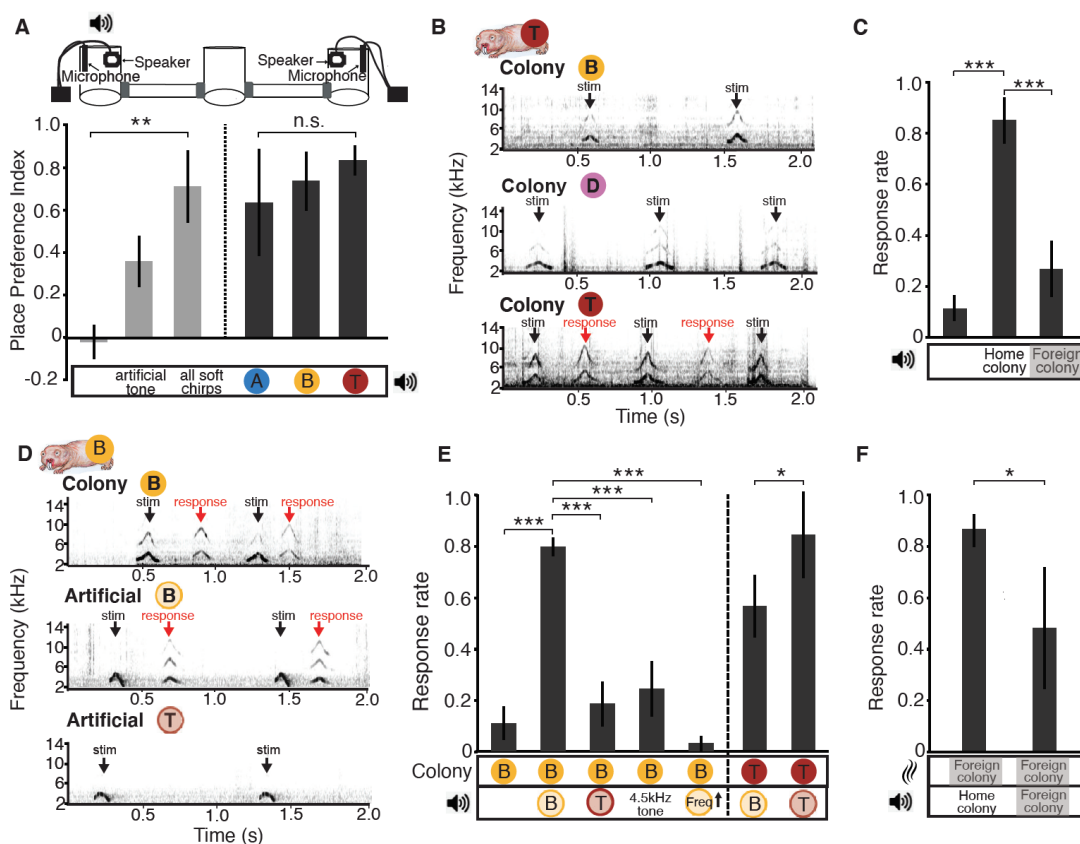


**Fig. 1. Naked mole-rat soft chirps encode individual identity (A)** Soft chirp analysis and classifier training workflow. **(B)** Individuals can be identified with high accuracy using machine learning tools trained on vocal features (A).

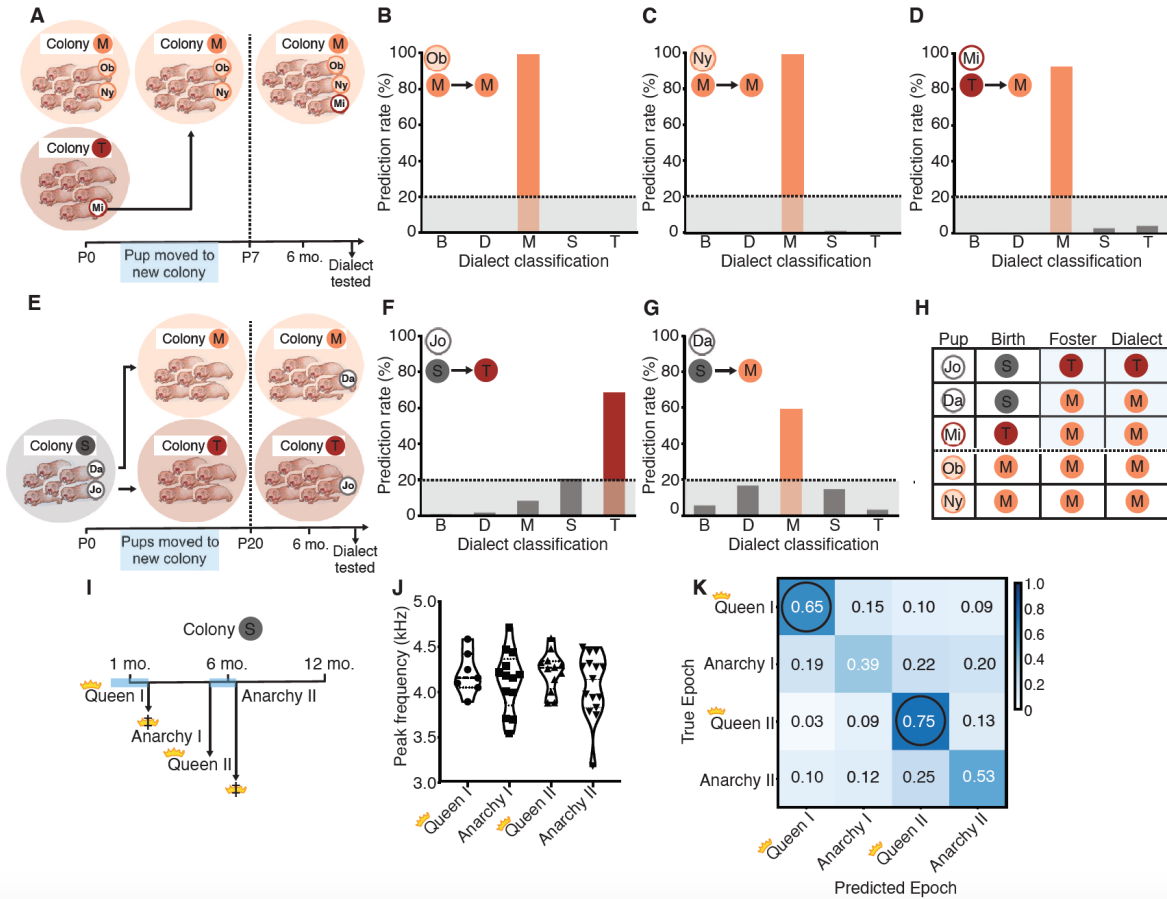




**Fig. 2. Naked mole-rat soft chirps signal colony identity.** (A) Schematic of classifier training. (B) Across four colonies (Colonies B, M and T; Berlin, Germany; Colony D; Pretoria, South Africa) colony identity is predicted with high accuracy. (C) Contributions of each vocalization feature to the colony classifier. (D) Asymmetry and peak frequency (inset in C) of soft chirps for all colonies. Error bars SEM.



**Fig.3. Vocal response rates are modulated by colony identity** (A) Left, in a Place Preference Assay naked mole-rats spend more time in the chamber with sound presentation compared to silence ( $n = 4$  animals;  $N \geq 36$  trials per animal, one-way ANOVA,  $P < 0.005$ ). Right, no place preference to colony-specific audio playbacks was observed. (B) Soft chirp response rates were enhanced to home colony audio playbacks. Example responses from Colony T animals. (C) Response rate is greater to home colony audio playback versus no playback or foreign colony playback, ( $n = 9$  animals;  $N \geq 36$ , one-way ANOVA,  $P < 0.0005$ ). (D) Example responses to home colony and artificial stimuli, Colony B. (E) For artificially generated stimuli, soft chirp responses to home colony classified audio playbacks are significantly increased compared to foreign colony classified audio playbacks or when frequency and asymmetry features alone are tested ( $n = 4$  animals, Colony B,  $n = 5$  animals, Colony T, one-way ANOVA or unpaired t test \*  $P < 0.05$ , \*\*  $P < 0.005$ , \*\*\*  $P < 0.0005$ ). (F) Colony-specific response rates were present when conflicting olfactory cues (bedding from a foreign colony) was placed in the test chamber. ( $n = 6$  animals,  $N \geq 36$  trials per animal,  $P < 0.05$ ). Error bars, SEM. For all experiments a minimum of  $N = 36$  behavioral trials performed per animal.



**Fig.4. Cultural transmission of colony dialects.** (A) Schematic of cross fostering. (B-D) Individual colony dialect predictions for each pup (non-fostered control pups, Ny and Ob and fostered pup Mi). Prediction accuracies: pup Mi = 95.5%, pup Ob = 99.2%, pup Ny = 99.0%). (E) Schematic of second cross-fostering. (F, G) Individual colony dialect predictions for foster pups Da and Jo (Da = 59.1%, pup Jo = 68.4% prediction rate for foster colony dialect). (H) All fostered pups adopt the dialect of their adoptive colonies. (I) Timeline of social upheaval in Colony S. J. During Anarchy periods variability in soft chirp frequency increases. (K). Colony classification accuracy decreases during anarchy periods compared to epochs with a stable queen (black circles).