

Interestingly, specific genes that had significantly altered expression in both cave populations had divergent patterns of expression, up in one population and down in the other, twice as often as convergent patterns, both up or both down. This suggests that the same systems are important for cave adaptation in the two populations but that they are modified in different, independently derived ways. We need to expand these studies to additional cave populations to determine the significance of this approach to the question of standing variation versus new mutation. These results, however, reinforce the conclusion that the end phenotype is important in cave adaptation, rather than the specific genetic changes involved.

In summary, cavefish bring the power of the replicated experiment to biology. Because each species of cave adapted fish has evolved independent of the others, they are replicates of the same natural experiment that asks, what happens when a surface population enters an environment with no light. Their study is expanding our understanding of the evolution, development, and key metabolic processes in the vertebrates at a great rate.

FURTHER READING

- Borowsky, R. (2008). Restoring sight in blind cavefish. *Curr. Biol.* 18, R23–R24.
- Casane, D., and Retaux, S. (2016). Evolutionary Genetics of the cavefish *Astyanax mexicanus*. *Adv. Genet.* 95, 117–159.
- Jeffery, W.R. (2009). Regressive evolution in *Astyanax* cavefish. *Annu. Rev. Genet.* 43, 25–47.
- Moran, D., Softley, R., and Warrant, E.J. (2015). The energetic cost of vision and the evolution of eyeless Mexican cavefish. *Sci. Adv.* 1, e1500363.
- Protas, M., Conrad, M., Gross, J.B., Tabin, C., and Borowsky, R. (2007). Regressive evolution in the Mexican cave tetra, *Astyanax mexicanus*. *Curr. Biol.* 17, 452–454.
- Protas, M., Tabansky, I., Conrad, M., Gross, J.B., Vidal, O., Tabin, C.J., and Borowsky, R. (2008). Multi-trait evolution in a cave fish, *Astyanax mexicanus*. *Evol. Dev.* 10, 196–209.
- Proudlove, G. cavefishes.org.uk.
- Stahl, B.A., and Gross, J.B. (2017). A comparative transcriptomic analysis of development in two *Astyanax* cavefish populations. *J. Exp. Zool.* 328, 515–532.
- Wilkens, H. (1988). Evolution and genetics of epigeal and cave *Astyanax fasciatus* (Characidae, Pisces) - support for the neutral mutation theory. *Evol. Biol.* 23, 271–367.
- Yang, J.X., Chen, X.L., Bai, J., Fang, D.M., Qiu, Y., Jiang, W.S., Yuan, H., Bian, C., Lu, J., He, S.Y., et al. (2016). The *Sinocyclocheilus* cavefish genome provides insights into cave adaptation. *BMC Biol.* 14, 1–13.

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Wild Sri Lankan elephants retreat from the sound of disturbed Asian honey bees

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Asian elephants (*Elephas maximus*) are threatened primarily by habitat loss and human–elephant conflict. In addition to establishing protected areas and corridors for wildlife, empowering farmers to protect their crops is crucial for Asian elephant conservation [1,2]. Elephants can habituate to artificial deterrents, hence natural biological alternatives are of great interest [2,3]. African elephants (*Loxodonta africana*) avoid African honey bees (*Apis mellifera scutellata*), inspiring ‘beehive fences’ as a successful means of small-scale crop protection [4,5]. Here, we used a recording of a disturbed hive of cavity-dwelling Asian honey bees (*Apis cerana indica*) and conducted sound playbacks to 120 wild elephants in 28 different groups resting under trees in Uda Walawe National Park in Sri Lanka. Elephants responded by moving significantly further away from their resting site in bee playback trials compared to controls. Elephants also increased vocalization rates, as well as investigative and reassurance behaviours in response to bee sounds, but did not display dusting or headshaking behaviour.

Our study focused on elephant responses to playbacks of *A. cerana indica* sounds primarily because it is the species most tractable for honey production in Asia. Although this Asian honey bee is smaller and appears less aggressive than its larger African cousin *A. mellifera scutellata*, it is morphologically similar, capable of stinging attacks, and contains a similar sized venom gland [6]. It therefore appears physically capable of causing discomfort to elephants. We completed 14 bee and 14 control playback trials using a control sound of natural white noise and recorded

responses and vocalizations from 120 known individual elephants representing a sample of between 10 and 15% of the total Uda Walawe elephant population [7]. Of these, 22 playback trials were to female groups/families (11 bee trials, 58 elephants: 4 trials at 15 m, 7 trials at 30 m; 11 control trials, 56 elephants: 4 trials at 15 m, 7 trials at 30 m). Six trials were to solitary bulls (3 bee trials: 1 trial at 15 m, 2 trials at 30 m; 3 control trials: 3 trials at 15 m). There were no differences in time of day, temperature, altitude, or air pressure between treatments (Mann-Whitney U tests all $p > 0.05$). (Supplemental information).

Elephants moved away more often in the bee trials (9/14) than in the control trials (4/14), although this difference was not statistically significant using Fisher’s exact test ($p = 0.128$). However, they moved significantly further away from bee sounds (mean distance $35.7 \text{ m} \pm \text{SE } 11.1$) than from control sounds ($8.2 \text{ m} \pm \text{SE } 3.3$; Mann-Whitney U test, $U = 56.5$; $p = 0.037$). The three bull elephants, upon hearing bee sounds, also moved further away on average ($55 \text{ m} \pm \text{SE } 24.66$) than the 11 female groups hearing bee sounds ($30.45 \text{ m} \pm \text{SE } 12.55$). Although the sample size precludes statistical testing, this trend is encouraging as bulls tend to be more conflict prone than females [2]. Groups bunched together significantly more in response to bee sounds (6/11) than to the control (1/11) (Chi-Square test, $X^2 = 5.24$, $df = 1$, $p = 0.022$). Elephants’ latency to move in response to bee sounds ($202.5 \text{ sec} \pm \text{SE } 41.22$) was shorter than that for control sounds ($289.71 \text{ sec} \pm \text{SE } 33.33$) but this difference was not significant (Mann-Whitney U test, $U = 63$, $p = 0.085$) (Figure 1).

During the playbacks, vocalizations were detected in recordings from 6/11 elephant groups hearing bee sounds but from only 2/11 groups hearing the control. We also observed 4 and 6 ‘trunk bounces’ to bee and control treatments, respectively, where elephants exhaled sharply whilst slapping the tip of the trunk onto the floor, a mildly agonistic behaviour unique to Asian elephants [8]. Groups hearing bee sounds ($n = 11$) showed significant differences in their vocalization rates between pre-stimulus, stimulus and post-stimulus phases of the playback trials with a peak of $0.36 (\pm \text{SE } 0.15)$ vocalizations per minute per elephant occurring during the



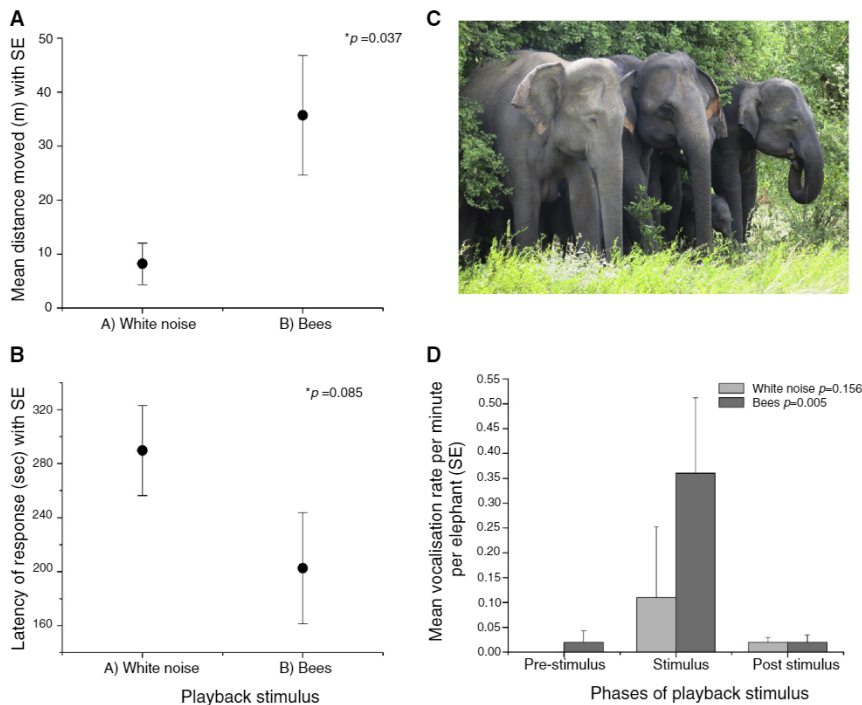


Figure 1. Behavioural data from the 28 playback trials.

Groups comprising both females and bulls hearing bee sounds (A) moved significantly further away and (B) moved away quicker/had a shorter latency of response than those elephants hearing the white noise control. (C) Asian Elephants resting and stationary under trees were ideal candidates for sound playbacks. (D) Elephant groups hearing bee sounds vocalized significantly more during the stimulus phase of the playbacks than either the pre-stimulus or post-stimulus phase ($F = 4.91$, $p = 0.005$). This was not the case for elephants hearing the white noise control who showed no significant difference between stimulus phases ($F = 0.72$, $p = 0.156$).

stimulus phase (Friedman's ANOVA, $N = 11$, $F = 4.91$, $p = 0.005$; Figure 1). Groups hearing white noise controls only vocalized $0.11 (\pm SE 0.14)$ times per minute per elephant during stimulus phase and vocalization rates did not vary significantly between phases (Friedman's ANOVA, $N = 9$, $F = 0.72$, $p = 0.15$). However, when we compared vocalization rates during just the stimulus phase between bees and control groups the difference was not significant (Mann-Whitney U Test, $U = 38.5$; $p = 0.115$). Bull trials were left out of this analysis as no vocalizations were recorded from any of the 6 individuals. Though the vocalization rates may not appear very high, Asian elephants in the wild very rarely vocalize at the observed frequency [8].

Behavioural responses during playbacks also notably differed between Asian and African elephants. We observed 31 incidents of trunk touching/placing of the trunk in a neighbour's mouth (27 in response to bee sounds, 4 in response to controls, $p = 0.062$), which

we interpreted as nervous behaviours of elephants seeking reassurance from a group member [9] and was not seen in African elephants responding to bees [4]. In our study there was some dusting behaviour but no significant difference in dusting events between bees ($n = 15$) and controls ($n = 4$). Notably there was no headshaking from any of the Sri Lankan elephants unlike in African elephants responding to bee stimuli [4] (Supplemental information). However, the gesture may differ functionally in the two species since headshaking is also not a commonly observed gesture in the Uda Walawe population, being most frequently associated with dominance behaviour [10].

Although some mixed results suggest further study would be valuable, this is the first investigation of how Asian elephants respond to a natural threat of disturbed honey bees. This study prompts further investigation of how Asian elephants would react if exposed to live honey bee stimuli.

SUPPLEMENTAL INFORMATION

Supplemental Information including experimental procedures and one table can be found with this article online at <https://doi.org/10.1016/j.cub.2017.12.018>.

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REFERENCES

1. Fernando, P., Jayewardene, J., Prasad, T., Hendavitharana, W., and Pastorini, J. (2011). Current status of Asian elephants in Sri Lanka. *Gajah* 35, 93–103.
2. Fernando, P., Kumar, M.A., Williams, A.C., Wikramanayake, E., Aziz, T., and Singh, S.M. (2008). Review of human–elephant conflict mitigation measures practiced in South Asia. AREAS Technical Support Document, World Wide Fund for Nature.
3. Thuphill, V., and Coss, R.G. (2013). Wild Asian elephants distinguish aggressive tiger and leopard growls according to perceived danger. *Biol. Lett.* 9, 20130518.
4. King, L.E., Douglas-Hamilton, I., and Vollrath, F. (2007). African elephants run from the sound of disturbed bees. *Curr. Biol.* 17, R832.
5. King, L.E., Douglas-Hamilton, I., and Vollrath, F. (2011). Beehive fences as effective deterrents for crop-raiding elephants: field trials in northern Kenya. *Afr. J. Ecol.* 49, 431–439.
6. PUNCHIHEWA, R.W.K. (1994). Beekeeping for Honey Production (Sarodaya Visha Lekha Publishing, Sri Lanka).
7. de Silva, S., Ranjeewa, A.D.G., and Weerakoon, D. (2011). Demography of Asian elephants (*Elephas maximus*) at Uda Walawe National Park, Sri Lanka based on identified individuals. *Biol. Conserv.* 144, 1742–1752.
8. de Silva, S. (2010). Acoustic communication in the Asian elephant *Elephas maximus*. *Behaviour* 147, 825–852.
9. Plotnik, J., and de Waal, F. (2014). Asian elephants (*Elephas maximus*) reassure others in distress. *PeerJ.* 2, e278.
10. de Silva, S., Schmid, V., and Wittemyer, G. (2017). Fission–fusion processes weaken dominance networks of female Asian elephants in a productive habitat. *Behav. Ecol.* 28, 243–252.

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