plained. The apparent warmth of Europe is accounted for by the poleward flow along the eastern margin of North America and through the poleward deflection of westward flow by the bulge of Africa. The mid-Pacific guyots are in a zone of westward flow and the Tethyan affinities could easily have a western Caribbean source. Finally, the model simulation provides opportunities for both east-to-west and west-to-east faunal migrations in Tethys. This result suggests that the biogeography, either because of an incomplete record or because the relations between biogeography and ocean circulation patterns are not fully understood, may not provide uniquely determined surface circulation patterns. Physically consistent and comprehensive reconstructions of ocean circulations, which can only be derived from fully resolved ocean circulation models, may be required to assess past ocean circulations.

The results of the numerical ocean model experiments for Cretaceous paleogeography indicate that the concept of a westward flowing circumglobal equatorial current for the Cretaceous and for much of the Mesozoic and Cenozoic must be re-assessed. At a minimum, earlier reconstructions are probably too simplistic and the circulation may have changed substantially as a function of ocean geometry (continental positions and sea level). The concept of poleward displacement of atmospheric winds during warm time periods, which has not been substantiated with GCM studies, apparently influenced earlier paleoceanographic reconstructions. Furthermore, ocean surface current reconstructions based on limited biogeographic data may not tightly constrain surface circulation patterns.

REFERENCES AND NOTES

1. B. P. Luyendyk, D. Forsyth, J. D. Phillips, *Geol. Soc. Am. Bull.* **83**, 2649 (1972).
2. W. A. Gordon, *J. Geol.* **81**, 269 (1973).
3. W. Berggren and C. Hollister, in *Studies in Paleo-Oceanography*, W. W. Hay, Ed. (*Spec. Publ. 20*, Society of Economic Paleontologists and Mineralogists, Tulsa, 1974), pp. 126–186.
4. F. C. Dilley, in *Faunal Provinces in Space and Time*, F. A. Middlemiss, P. F. Rawson, G. Newall, Eds. (Special Issue 4 of *Geological Journal*, Seel House Press, Liverpool, 1971), pp. 169–190.
5. W. R. Holland, *Sea* **6**, 3 (1977).
6. K. Bryan, *Dyn. Atmos. Oceans* **3**, 327 (1979).
7. D. G. Seidov and V. K. H. Yenikeyev, *Oceanology* **24**, 495 (1984).
8. J. Sundermann and S. Legutke, *Geol. Rundsch.* **75**, 105 (1986).
9. The details of the ocean model are given by A. J. Semtner, *Univ. Calif. Los Angeles Meteorol. Tech. Rep. 9* (1974). Examples of modern-day simulations are described by G. A. Meehl, W. M. Washington, A. J. Semtner, *J. Phys. Oceanogr.* **12**, 301 (1982), and W. M. Washington, A. J. Semtner, G. A. Meehl, D. J. Knight, T. A. Mayer, *J. Phys. Oceanogr.* **10**, 1887 (1980).
10. K. Bryan, *J. Comput. Phys.* **3**, 347 (1969).
11. E. J. Barron, C. G. A. Harrison, J. L. Sloan, W. W. Hay, *Ecologae Geol. Helv.* **74**, 443 (1981).
12. E. J. Barron, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **59**, 3 (1987).
13. ——— and W. M. Washington, *J. Geophys. Res.* **9**, 1267 (1984).
14. ———, in *The Carbon Cycle and Atmospheric CO_2: Natural Variations Archean to Present*, E. T. Sundquist and W. S. Broecker, Eds. (American Geophysical Union, Washington, 1985), pp. 546–553.
15. ———, *Geology* **10**, 633 (1982).

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Hearing in Honey Bees: Detection of Air-Particle Oscillations

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Although the airborne sounds produced by dancing honey bees seem essential in the bees' dance communication, attempts to show directly that bees can detect airborne sounds have been unsuccessful. It is shown here that bees can in fact detect airborne sounds and that they do so by detecting air-particle movements. Most vertebrates, by contrast, detect pressure oscillations. Because all traveling sound waves have both components, either can be used in sound detection. The bees' acoustic sense appears to be sensitive enough to allow bees to detect the air-particle movements that occur within several millimeters of a sound-emitting dancer.

ALTHOUGH MUCH IS KNOWN ABOUT the orientation of honey bee (*Apis mellifera*) dancers and the role of the dance language in a colony's overall biology, we still do not know through which sensory modalities the dance communication occurs (*1*). Sounds, however, are evidently essential: dancers produce sounds by vibrating their wings (*2*), and the dances of bees with clipped wings and of the mutant diminutive wings are ineffective (*3*), as are silent dances by bees with normal wings (*4*). Further, a mechanical model of a dancing bee can successfully communicate with its "nestmates" only when its artificial wings generate normal sounds (*5*). The vibrations of a dancer's wings generate strong air-particle oscillations within a few millimeters of the dancer's abdomen, but only relatively weak pressure oscillations (*2, 6*) and no comb vibrations (*7*), suggesting that the sounds may be detected by dance followers as air-particle movements (*8*). Uninformed by these insights, previous attempts to elicit responses by bees to airborne sounds have been unsuccessful (*9*). Here we report our efforts to condition bees to respond to artificial airborne vibrations similar to those produced by dancers.

Individual bees visiting a feeder were trained to associate the presentation of a 5-s-long sound (conditioned stimulus, CS) with a mild electric shock. On each training trial, the shock was delivered 4 s after the sound's onset and caused the bee immediately to withdraw from the feeder (*10*). The sounds were generated at the open end of a narrow glass tube by driving the air column in the tube with a loudspeaker (*11*). The open end of such a tube is a site of strong air-particle oscillations and relatively weaker pressure oscillations (*6*). Two different frequencies of sound were used as CS at different times: 14 Hz, the frequency of a dancer's abdominal waggling, and 265 Hz, the frequency of the dance sounds (*12*). On each training trial, the bee was scored as responding to the sound if she withdrew from the feeder in the 4-s CS-only interval, before the shock.

Twenty-four bees were individually trained to withdraw from the feeder in response to one of the sound frequencies (CS+) until each bee responded positively on at least five out of ten sequential trials (*13*). Sixteen of these trained bees, eight with 265 Hz as CS+ and eight with 14 Hz as CS+, were then used in a 20-trial frequency-discrimination procedure. In this procedure, each bee was given her CS+ and the alternative frequency (CS−) for ten trials each in pseudorandom order. The results, shown in Fig. 1, indicate that the bees can detect and learn to respond to both frequencies (acquisition, left) and that they can discriminate each frequency from the other (discrimination, right).

Because bees are highly sensitive to substrate vibrations, such vibrations in these experiments were measured and held below the bees' known physiological thresholds (*14*). Furthermore, eight additional bees trained to associate the 265-Hz sound with shock

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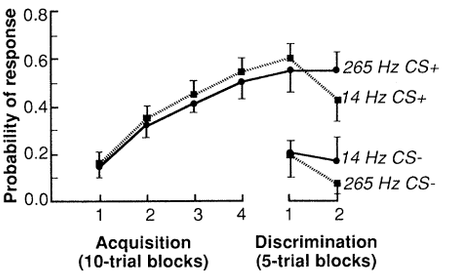

Fig. 1. Aversive conditioning to airborne vibrations. For acquisition, the probability of response to the CS+ frequency is plotted in ten-trial blocks for both groups of bees (265-Hz CS+ and 14-Hz CS+). For discrimination, the probabilities of response to both CS+ and CS− frequencies are plotted in five-trial blocks for each group (bees in the same group are indicated with like symbols). The bees in each group responded significantly more to their respective CS+ than CS− frequencies (t tests, $P < 0.01$ in each case). Bars represent ± SEM; $n = 8$ bees in each group. Block 4 of the acquisition contains not only trials 31 to 40 but also trials 41 to 50 for those bees that needed 50 trials to reach the 50% response level (*22*).

were observed as they approached a feeder at which the sound was left on continuously as they arrived. In this situation, the trained bees hovered briefly about 1.5 cm from the sound-emitting tube, departed, and approached again repeatedly before landing. The same bees showed no such hesitancy in landing when they arrived and found the sound off. The mean interval between initial approach and landing was 26 (±14) s with the sound on and 2.3 (±2) s with the sound off ($n = 80$ in each case; all values here and below are given with ±1 SD). Thus these sounds can be detected by airborne bees, indicating that they are received through the air (and separately from whatever sounds the flying bee herself makes).

To determine whether the bees respond to the pressure or particle-movement component of the 265-Hz sound—all traveling sound waves have both components (*6*)—individual bees were trained to visit a feeder inside a large standing-wave tube. Within such a tube there are sites of maximal particle movement (with minimal pressure oscillation) and, elsewhere, sites of maximal pressure oscillation (with minimal particle movement) (*15*). Each of 12 bees was stimulated ten times at a pressure maximum and ten times at a particle-movement maximum (*16*) and the bees' responses videotaped. Analysis of the tapes showed that the bees gave two types of response, lowering of the wings and raising of the antennae, but only at the particle-movement maximum. The overall probability of responding on a given trial at the particle-movement maximum was 0.79 (±0.3) for the wings and 0.49 (±0.3) for the antennae ($n = 12$ bees) (*17*). At the pressure maximum, the probabilities for both responses were 0.02 (±0.04) (*18*). Thus we regard particle vibration as the adequate stimulus.

Next, several naïve bees were stimulated at the particle-movement maximum inside the large standing-wave tube with particle oscillations of the same amplitude as those found within a few millimeters of a dancing bee (*19*). The bees responded [response rates, 0.44 (±0.3) for the antennae and 0.64 (±0.3) for the wings; $n = 9$ bees], indicating that dance followers within a few millimeters of a dancer can probably detect the air-particle oscillations in the natural dance signals. In addition, 22 bees with their wings clipped off were stimulated once each at a peak-to-peak particle displacement of 1.4 mm. Their antennal response rate (0.64) was not significantly different from that of bees with intact wings given the same stimulus [response rate, 0.73 ± 0.3; $n = 9$ bees; $P > 0.5$, $t(8) = 0.28$]. Thus the antennal reaction is induced by receptors other than those associated with the wings, perhaps receptors in the antennae themselves.

We also tried to condition bees to associate the 265-Hz stimulus inside the large standing-wave tube with an electric shock. The bees failed to learn, even when the particle movements inside the large tube matched in frequency and amplitude those with which we successfully trained bees at the open end of the narrow tube (first experiments above). One possible explanation for this is that the air movements at the end of the narrow tube contained direct current components not present inside the large tube. These local air streams resulted from circular currents flowing outward along the narrow tube's long axis and returning radially inward around the tube's lip (*20*). Whether or not the direct current components are important here and whether or not the natural dance signals have similar components are both unknown. However, the ability of the bees to discriminate the 14-Hz stimuli from the 265-Hz stimuli in the training experiments and the reflexive reactions of the bees inside the large tube both indicate that our bees have responded to the vibratory components of the stimuli, not only (if at all) the direct current components.

A second difference between the stimuli in the two situations was that the particle movements at the open end of the narrow tube (where the bees learned) declined rapidly in amplitude with distance from the source; inside the large tube, by contrast, the sound field was much more spatially uniform. This suggests that bees may filter out spatially uniform signals, which would help them to respond selectively to the spatially varying near-field signals of dancers (*2, 6*), ignoring background noise from distant sources. This would require two receptors spatially separated from one another.

The most likely receptors of the air-particle oscillations appear to be the Johnston's organs, which are located at the base of each antennal flagellum and respond best to air movements of 250 to 280 Hz, the frequency of the dance sounds (*21*). Being bilateral, these organs could yield information on a dance attender's spatial orientation relative to that of the dancer, which, in turn, would indicate the direction of the food source being advertised (*2*).

REFERENCES AND NOTES

1. W. F. Towne, *Behav. Ecol. Sociobiol.* **16**, 185 (1985).
2. A. Michelsen *et al.*, *J. Comp. Physiol.* **161**, 633 (1987).
3. W. H. Kirchner, in preparation.
4. H. Esch, *Verh. Dtsch. Zool. Ges.* **1962**, 302 (1963). Such silent dances occur most often when the food source being advertised is of low quality.
5. A. Michelsen, B. B. Anderson, W. H. Kirchner, M. Lindauer, in preparation.
6. Far from a sound source (in the acoustic far field), air-particle velocity, v, and sound pressure, p, are related by the equation $v = p/\rho c$, where ρ is the density of air and c is the propagation velocity of sound waves in air. Nearer the sound source (in the near field), this relation no longer holds; the particle velocity becomes higher relative to the pressure ($v > p/\rho c$), more so the closer one approaches the source. The bees' dance communication occurs within the extreme near field (where $v >> p/\rho c$) (*2*), so in these experiments we used stimuli whose particle movements were strong relative to their pressures.
7. A. Michelsen, W. H. Kirchner, M. Lindauer, *Behav. Ecol. Sociobiol.* **18**, 207 (1986).
8. J. Tautz [*Naturwissenschaften*, **66**, 452 (1979)] reviews the abilities of arthropods to detect air-particle oscillations.
9. A. Hansson, *Opusc. Entomol.* (1945), suppl. IV, p. 1; A. M. Wenner, *Anim. Behav.* **10**, 79 (1962); E. K. Es'kov, *Probl. Peridach. Inform.* **8**, 83 (1972) [in Russian].
10. The shock was a 20 ms, 60 V, direct current pulse delivered through two electrodes, one attached to the platform on which the bees stood and the other in contact with the sugar-water solution the bees collected. Method after C. I. Abramson [*J. Comp. Physiol. Psych.* **100**, 108 (1986)].
11. The tube had an inner diameter of 11 mm and was 64 cm long. It was connected to a wooden box containing the speaker by a funnel-shaped adaptor. During stimulation, the bees positioned their heads about 6 mm from the open end of the tube.
12. The air column in the tube resonated at 265-Hz, which increased the amplitude of the stimuli that could be generated at that frequency. The 14-Hz stimulus involved no resonance; the air column was driven directly by the speaker. The sound pressures and particle-movement amplitudes where the bees stood were as follows: for the 14-Hz stimulus, 4-Pa (root-mean-square) sound pressure and 0.8-m/s peak particle velocity; and for the 265-Hz stimulus, 10-Pa (r.m.s.) sound pressure and 2.8-m/s peak particle velocity. Both stimuli had gradual (1 s) onsets and offsets.
13. Most bees required between 30 and 50 trials to reach the 50% response criterion. Seven additional bees were trained but either failed to respond at all in the first 20 trials or failed to reach the 50% criterion in 50 trials. In either case, the bees were captured and dropped from the experiments.
14. H. Autrum and W. Schneider, *Z. Vergl. Physiol.* **31**,

77 (1948).
15. Method after H. Autrum [*Z. Vergl. Physiol.* **23**, 332 (1936)] and H. Markl and J. Tautz [*J. Comp. Physiol.* **99**, 79 (1975)]. These authors describe the acoustics of closed tubes like the one we used and illustrate how such tubes can be used in the analysis of insect hearing. Our tube had an inner diameter of 9.5 cm and was plugged on each end by a wooden piston, one of which contained an 8.5 cm dynamic loudspeaker. The bees gained access to the feeder inside the tube through a 3-cm hole that was plugged after the bee entered.
16. The tones were 3 s long, including gradual (1 s) onsets and offsets. The sound pressure and particle-movement amplitudes at the two sites were as follows: at the pressure maximum, 60-Pa (r.m.s.) sound pressure and 0.07-m/s peak particle velocity; and at the particle-movement maximum, 18-Pa (r.m.s.) pressure and 1.0-m/s peak particle velocity.
17. These were clearly not passive movements forced by the air oscillations. The antennae were moved over 2 mm upward and outward and the wings strongly retracted. The peak displacement of the air particles, for comparison, was 0.6 mm.
18. These response rates, each of which represents two responses out of 120 trials, probably reflect the "misfire" rate, the rate at which spontaneous movements of the bees happened to coincide in time with the sound and were therefore scored as positive responses.
19. The peak particle velocity within a few millimeters of a dancing bee is about 0.7 m/s (*2*).
20. We detected these currents with a small (3 mm) hair placed in the sound field and observed through microscope optics. The hair not only oscillated at the frequency of the sound but also was deflected by the currents.
21. H. Heran, *Z. Vergl. Physiol.* **42**, 103 (1959).
22. The bees that never reached the 50% response level are excluded (*13*) and did not undergo the discrimination procedure.
23. We thank D. R. Griffin for advice and technical help throughout, A. Michelsen and C. Sherrick for several useful suggestions and help in calibrating our transducers, M. Lindauer and J. L. Gould for encouragement and logistical support, F. C. Dyer for suggesting that we train bees with sounds and M. E. Bitterman that we try the Abramson technique, and C. Ristau and others for reviewing the manuscript. Supported by NSF grant BNS 85-09522 to J. L. Gould, and a grant from The Volkswagen Foundation to M. Lindauer.

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Fibroblast Growth Factor in the Extracellular Matrix of Dystrophic (mdx) Mouse Muscle

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Polyclonal antibody F547 reacts with a bovine basic fibroblast growth factor (bFGF) and a human recombinant bFGF, but not with bovine acidic fibroblast growth factor. This antibody localized bFGF in the extracellular matrix of mouse skeletal muscle, primarily in the fiber endomysium, which includes the heparin-containing basal lamina. In mdx mouse muscle, which displays persistent regeneration, FGF levels in the extracellular matrix are higher than those in controls. Overabundance of matrix FGF in mdx muscles may be related to an increase in both satellite cell and regenerative activity in the dystrophic muscle and may help explain the benign phenotype of mdx animals compared with the genetically identical human Duchenne muscular dystrophy.

Skeletal muscle regeneration depends on the presence of satellite cells embedded in the heparin-rich basal lamina of individual muscle fibers (*1*). During growth and maturation, and after injury in the adult, these stem cells are activated by unknown mechanisms, initiate cell division, and divide to generate a population of myoblasts that fuse to existing fibers to maintain constant ratios of nuclei to cytoplasm (during maturation) or fuse to form regenerated fibers (after adult injury) (*2*). Muscle myoblasts and satellite cells respond to FGF (*3*) and to other mitogens (*4*), and various muscles store an FGF-like activity (*5*). In addition, FGF and other growth factors bind strongly to heparin (*6*); FGF stimulation of myogenic cells is inhibited by heparin in cell cultures of both embryonic myoblasts and adult satellite cells (*7*). The negative regulation of satellite cell growth may therefore be related to heparin-FGF binding in the heparin-rich muscle basal lamina, and the positive response after injury may be related to a disruption of the heparin-FGF association (*8*).

However, to our knowledge, FGF has not been found in muscle basal lamina in vivo. Because of the persistent regeneration of mdx mouse muscle in comparison with its congenic normal strain, we looked for differences in FGF localization in muscle tissue. The mdx mouse (*9*) has the phenotype of a mild muscular dystrophy (*10*) but has the same X-chromosome gene mutation as the one responsible for Duchenne muscular dystrophy in humans and that is linked to an absence of the protein dystrophin within muscle fibers (*11*). Mdx fibers also lack dystrophin (*12*) but somehow escape the fatal phenotype characteristic of the human condition (*10, 13*). In normal mouse skeletal muscle, bFGF is localized around individual muscle fibers in areas identified as the fiber endomysium, the innermost component of which is the basal lamina. Moreover, in muscles from mdx animals the concentration of endomysial-bound FGF appeared amplified. The increase of FGF in the extracellular matrix of mdx muscles correlated with an increased regenerative activity in the muscles, which persists well into adult age (*10*).

We isolated and purified bovine bFGF by heparin-Sepharose chromatography (*6*). The purified protein was used as antigen to produce a polyclonal antibody to FGF (F547) in mice. It specifically stained bFGF but not bovine acidic FGF (aFGF) in an immunoblot (Fig. 1). This antibody also cross-reacted with human recombinant bFGF (Fig. 1) but not with other minor contaminants in our antigen preparation, and excess bFGF completely inhibited antibody reaction in frozen sections (Fig. 2). Thus, the antibody appears to be specific for bFGF.

Frozen sections of normal hind limb muscle that were stained with antibody F547 were examined microscopically (Fig. 2). All muscles were from 10-week-old animals. In the mdx mouse, the major burst of degeneration and regeneration takes place at 5 to 8 weeks (*10*), so that at 10 weeks the muscle has completed this initial regenerative re-

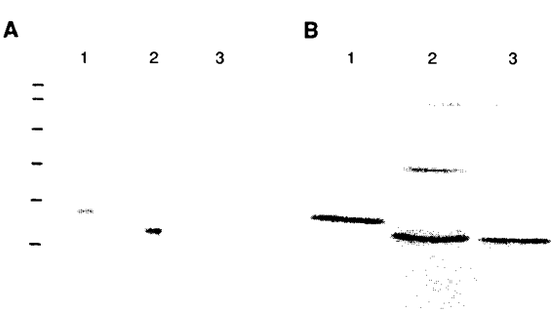

Fig. 1. (**A**) Immunoblot analysis of FGF preparations with antibody F547. The proteins were separated on a 12% SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with F547 at a dilution of 1:200. Binding was visualized with a horseradish peroxidase staining kit (Vector Labs.). Protein molecular size markers (horizontal bars on the left) (Pharmacia LKB) are 94, 67, 43, 30, 20.1, and 14.4 kD. Lane 1, human recombinant bFGF, a full-length unmodified peptide; lane 2, bovine bFGF; lane 3, bovine aFGF. The bFGF and aFGF eluted at concentrations of 2*M* and 1*M* NaCl, respectively, from heparin-Sepharose affinity columns as described (*6*). Antibody F547 did not stain either laminin or fibronectin on similar protein immunoblots (*17*). (**B**) Stained gel of protein samples shown in (A). Stain was FAST-STAIN (Zoion Research, Allston, Massachusetts). Lane contents and electrophoresis conditions (*18*) were identical to those in (A).

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