DNA transfer from the bacterium to the plant cell and the subsequent steps involve the *vir*E gene product(s) and the integration of T-DNA into plant host chromosomes.

Experiments in which *Agrobacterium* is cocultivated with regenerating plant protoplasts show that this bacterium is a highly efficient vector for DNA transfer, giving transformation rates of up to 50 percent (*18*). The availability of mutants of *Agrobacterium* that efficiently transfer DNA into plant cells, but do not integrate the DNA, might provide a means for the isolation or identification of autonomously replicating sequences or of centromeres from plants. *Agrobacterium* also has the potential to become a "suicide" vector for the introduction of transposable elements into plants or for the assessment of plant transformation systems on the basis of homologous recombination.

REFERENCES AND NOTES

1. E. W. Nester and T. Kosuge, *Annu. Rev. Microbiol.* **35**, 531 (1981).
2. V. N. Iyer, H. J. Klee, E. W. Nester, *Mol. Gen. Genet.* **188**, 418 (1982).
3. R. F. Barker, K. B. Idler, D. V. Thompson, J. D. Kemp, *Plant Mol. Biol.* **2**, 335 (1983).
4. C. H. Shaw, M. D. Watson, G. H. Carter, C. H. Shaw, *Nucleic Acids Res.* **12**, 6031 (1984).
5. K. Wang, L. Herrera-Estrella, M. Van Montagu, P. Zambryski, *Cell* **38**, 455 (1984).
6. D. J. Garfinkel and E. W. Nester, *J. Bacteriol.* **144**, 732 (1980).
7. G. Ooms, P. M. Klapwijk, J. A. Poulis, R. A. Schilperoort, *ibid.*, p. 82.
8. H. J. Klee *et al.*, *ibid.* **153**, 878 (1983).
9. R. Gardner, K. R. Chonoles, R. A. Owens, *Plant Mol. Biol.*, in press.
10. D. E. Cress, M. C. Kiefer, R. A. Owens, *Nucleic Acids Res.* **11**, 6821 (1983).
11. M. Tabler and H. L. Sanger, *EMBO J.* **3**, 3055 (1984).
12. A. Hoekema *et al.*, *Nature (London)* **303**, 179 (1983).
13. N. S. Yadav *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6322 (1982).
14. P. Zambryski, A. Depicker, K. Kruger, H. Goodman, *J. Mol. Appl. Genet.* **1**, 361 (1982).
15. Z. Koukolikova-Nicola *et al.*, *Nature (London)* **313**, 191 (1985).
16. V. C. Knauf and E. W. Nester, *Plasmid* **8**, 45 (1982).
17. L. Otten *et al.*, *Mol. Gen. Genet.* **195**, 159 (1984).
18. R. T. Fraley *et al.*, *Plant Mol. Biol.* **3**, 371 (1984).
19. R. A. Owens and T. O. Diener, *Science* **213**, 670 (1981).
20. These experiments were reviewed by an institutional biosafety committee prior to initiation of the investigations; all plant material was contained in a single committed growth chamber and was autoclaved after analysis.

21. We thank J. Hille, who suggested analogous experiments with the cauliflower mosaic virus; R. Owens and R. Goodman, who proposed the use of *Agrobacterium* to transfer cDNA copies of viroids; and those colleagues who reviewed this manuscript before submission. We thank R. A. Owens for PSTV cDNA clones, K. R. Chonoles for technical assistance, and L. Comai and B. Rose for some of the border-containing plasmids.

16 May 1985; accepted 5 November 1985

The Cyclopean Ear: A New Sense for the Praying Mantis

DAVID D. YAGER AND RONALD R. HOY

The praying mantis, thought to be deaf, possesses a sensitive and specialized acoustic sense. Neural recordings show that the auditory system responds primarily to ultrasound between 25 and 45 kilohertz with thresholds of 55 to 60 decibels. Other insects with auditory tympana possess paired, laterally placed ears; the mantis has only a single ear that is located in the ventral midline between the metathoracic legs. Some species of mantis abruptly and dramatically alter their flight path when stimulated with ultrasonic pulses, suggesting a behavioral response to insectivorous echo-locating bats.

ALL ANIMALS THAT HEAR USING true ears have two functional hearing organs. Tympanal auditory organs occur in members of four insect orders (*1*) and in all cases the ears are widely separated on the body allowing maximum opportunity for these small animals to obtain directional information about the sound source (*2*). We report tympanate hearing in another insect order, the Dictyoptera (mantises, cockroaches, and termites). Moreover, unlike other animals, the praying mantis possesses a single ear located in the ventral midline: it is an auditory cyclops.

Our evidence for audition in the praying mantis, *Mantis religiosa,* is first based on extracellular neural responses recorded from the ventral nerve cord of wild-caught adults (*3*). Stimulation with sound over a broad range of frequencies and intensities elicited strong stimulus-locked responses. At most, four action potential size classes could be distinguished. In the best frequency range for the response, the shortest latency to the first spike was 10 to 14 msec. The composite response was tonic, persisting throughout a 300-msec tone burst, and showed very high initial spike rates (up to 750 spikes per second) followed by instantaneous rates of 150 to 400 spikes per second.

The frequency tuning of the overall extracellular response is shown in Fig. 1 (solid line). These mantises hear best in the ultrasonic range. They are at least 30 times less sensitive at frequencies below 10 kHz as they are in their range of maximum sensitivity: 25 to 45 kHz. The shape of the mantis auditory tuning curve is similar to those of lacewings, many moths, and to that of an interneuron important in the cricket auditory system (*4*). In the best frequency range, the mantis hearing sensitivity [55 to 60 dB SPL (sound pressure level)] is comparable to those of lacewings and crickets (50 to 60 dB SPL), though less than that of moths (40 to 45 dB SPL).

We were not able to show any directional component in the auditory response. Sound stimuli at 35 kHz presented from either the side ipsilateral or contralateral to the recording electrode elicited an identical neural response as long as the sound pressure level of the stimuli from each side, measured at the animal, was the same.

By intracellular recording in the metathoracic connective (*5*), we discovered two types of cells that gave strong responses to sound. One of these types was penetrated twice, but only briefly. It was characterized by a best frequency range of 20 to 40 kHz, latencies of 20 to 30 msec, best sensitivities of 55 to 60 dB SPL, and a phasic firing pattern: over a broad stimulus intensity range it responded with only one to two spikes at the stimulus onset.

The second cell type encountered with intracellular recording showed temporal and frequency response patterns closely resembling the extracellular recordings. The tuning curve for one of these cells is shown in Fig. 1. It differs from the extracellular curve only at the low and high extremes of the frequencies tested. Both the latency and the number of spikes per stimulus varied with intensity in exactly the same manner as those for the extracellular response. This cell type fired tonically throughout a 300-msec tone burst; the initial rates of 550 to 600 spikes per second drop after 3 to 5 spikes to a steady rate of 100 to 200 spikes per second.

Figure 1 also shows the anatomy of one of these tonically firing auditory interneurons. It has a large (7 to 12 μm) ascending axon that lies in the dorsolateral quadrant of the connective near the sheath. Processes of this cell branch widely in the hemiganglion ipsilateral to the axon, and a major branch also crosses the midline. The soma was not filled in any of our preparations.

Transections at several levels of the ventral

Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

nerve cord established that the auditory response originated in the metathorax. The neural response remained unchanged after amputation of all six legs at the base of the coxa (*6*) and after covering all of the thoracic spiracles (in fact, the entire dorsal and lateral surfaces of the animal) with a heavy layer of Vaseline (*7*).

We discovered, however, that in a completely intact mantis, a small drop of melted wax placed over a deep, narrow groove located in the midline between the metathoracic legs entirely eliminated the auditory response. Therefore, this groove was necessary for hearing. In other animals we removed all legs, covered the animal except for the ventral-most surface in the area of the groove with Vaseline, and transected the abdomen at the first abdominal segment. Hearing remained intact, however, until we also covered the groove; this manipulation raised the threshold by more than 25 dB. Thus we found that a single, midline structure—the 'ear'—was both necessary and sufficient for hearing in the mantis.

The area of the metathoracic groove shows several specializations consistent with its role in hearing (Fig. 2) (*1*). As in other insect tympana, the cuticle of both lateral walls of the metathoracic groove is thinned, especially so in a longitudinal depression shaped like an elongated teardrop. Closely opposed to each lateral wall of the groove on its internal side is a large tracheal sac, one of the three stacked behind each wall. The sacs arise by narrow branches off the main tracheal commissure from the first abdominal spiracle.

We discovered a small neural structure that may contain the primary auditory sensory neurons attached to the anterior-medial border of the largest air sac on each side. As in the tympanal end organs of other insects (*1*), the structure contains nerve cell bodies, many smaller nuclei, and scolopale caps and rods. It is associated with a ligament anchored to the ventral body wall and near the border of the thinned cuticle (*8*). Innervation is supplied by a nerve containing approximately 20 axons, 3 to 5 μm in diameter, as well as other, much smaller fibers. The nerve travels to the metathoracic ganglion where it enters slightly anterior and ventral to the main leg nerve.

The single ear of the mantis comprises two tympana facing each other in a deep cleft and separated by less than 150 μm. Functionally this suggests that the ear may be unable to provide directional information, a possibility that our data support (*9*). Sound shadowing of very high frequencies by the thorax could, however, provide a basis for localization if accompanied by rotational scanning motions of the body.

Our discovery of hearing in mantises suggests that they possess a more complex behavioral repertoire than previously appreciated. We do not yet know what role detection of ultrasonic signals plays in the natural history of mantises. Perhaps weak, ultrasonic signals are produced during courtship. The rotatory abdominal motions reported by Liske and Davis (*10*) in *Tenodera* courtship could produce such sounds as the abdomen rubs the wings. We know that some mantises are not strictly diurnal (*11*). Males fly after dark following pheromone trails produced by females; courtship and mating occur during the night or near dawn. Perhaps, by analogy with flying moths, lacewings, and crickets, flying mantises listen to the ultrasonic biosonar signals of insectivorous bats (*12*). Our preliminary experiments show that an Asian hymenopodid mantis responds to batlike ultrasound pulses with a sudden full extension of its forelegs and a strong dorsiflexion of the abdomen. The response occurs only when the animal is in flight and causes an abrupt and dramatic deviation in its flight path. The mantis thus has independently evolved not only a novel ear but possibly a complex nocturnal predator avoidance system.

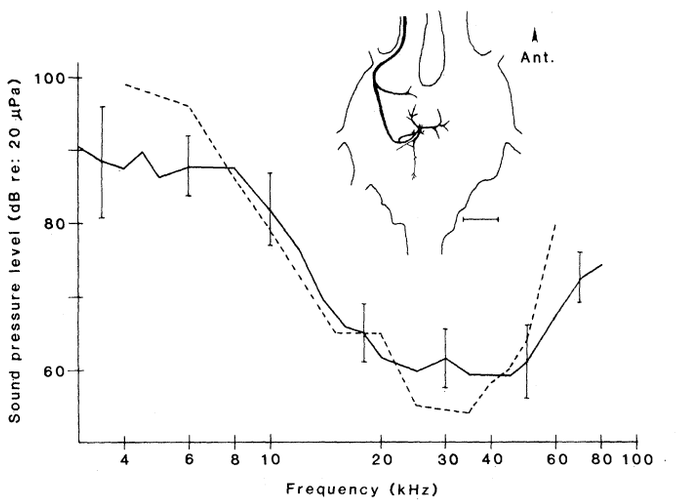

Fig. 1 (left). The graph shows the frequency tuning of the neural auditory response of *Mantis religiosa*. The solid line is the mean tuning curve (vertical bars, ±1 standard deviation) obtained in extracellular recordings from seven males and six females. (Tuning curves for the two sexes were the same.) The lowest threshold observed extracellularly was 49 dB SPL at 35 kHz. The dashed curve shows the frequency tuning of a single, tonically firing auditory interneuron recorded intracellularly. The four cells of this type that we encountered had virtually identical tuning curves and similar anatomy. The inset shows the anatomy of one of these cells in the metathoracic ganglion drawn from photographs and camera lucida sketches of the Lucifer yellow–filled preparation. Scale bar, 200 μm.

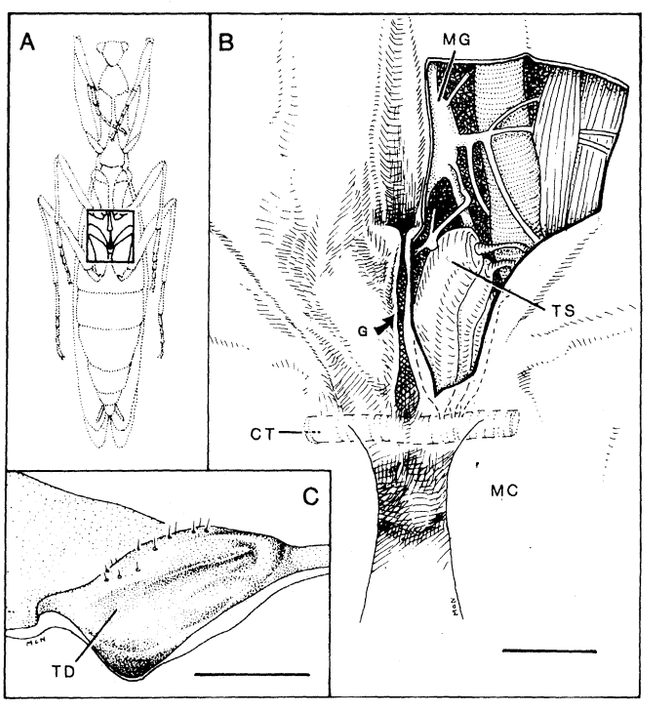

Fig. 2 (right). (A) Ventral view of female mantis. (B) The box in (A) outlines the region surrounding the ear that is enlarged in (B). The arrow (G) points to the deep groove containing the tympana. Cuticle, the underlying fat body, and some tracheae have been removed on the animal's left side. The tracheal sacs (TS), arising from commissural trachea (CT) near the base of the metathoracic coxa (MC), have been deflected slightly away from the tympanum to expose fully the neural structure attached anteriorly. The tympanal nerve travels without branching to the metathoracic ganglion (MG). The suspensory ligament remains fixed at the tympanum, but the end normally attached to the ventral cuticle is free. Scale bar, 1 mm. (C) Mid-sagittal view of the right tympanum showing the elongated teardrop-shaped depression (TD). Anterior is to the right. Scale bar, 1 mm.

REFERENCES AND NOTES

1. A. Michelsen, *Am. Sci.* 67, 696 (1979); M. Hutchings and B. Lewis, in *Bioacoustics—A Comparative Approach,* B. Lewis, Ed. (Academic Press, New York, 1983). The ordinal divisions of the orthopteroid insects have been a matter of some debate. We follow the classification scheme given in H. Evans, Ed. [*Insect Biology—A Textbook of Entomology* (Addison-Wesley, Menlo Park, NJ 1984)].
2. A. Michelsen and H. Nocke, *Adv. Insect Physiol.* 10, 247 (1974).
3. Animals were waxed ventral side up to a platform, the legs were removed, and a small rectangle of cuticle was lifted from the ventral mesothorax. The preparation was then moved to the center of a 0.7 by 2.0 m chamber lined with acoustic foam. A suction electrode was placed over the cut end of the connective, usually just caudal to the prothoracic ganglion. Sound stimuli (30 to 300 msec long; 5-msec rise and fall) were produced by a Tektronix FG501 function generator and a homemade trapezoid generator. We calibrated and analyzed the output of speakers using a quarter-inch B&K microphone (grid off) on a B&K 2209 Impulse SPL meter and a Nicolet MiniUbiquitous FFT spectrum analyzer.
4. A. Moiseff and R. R. Hoy, *J. Comp. Physiol.* 152, 155 (1983); J. H. Fullard and R. M. Barclay, *Can. J. Zool.* 58, 1745 (1980); L. A. Miller, *J. Insect Physiol.* 17, 491 (1971).
5. Animals were prepared as described in (3) except that a small square of cuticle was removed to expose the connectives between the meso- and metathoracic ganglia. We used glass microelectrodes filled with 5 percent Lucifer yellow CH in 1.0*M* $LiCl_2$ (resistance: 130 to 180 megohms) and standard recording electronics.
6. The auditory organs of the tettigoniids and gryllids are on the tibia (1). Most insects also have in their legs subgenual organs that respond to substrate vibration and low frequency sound. We found that the mantis subgenual response had thresholds as low as 45 to 50 dB SPL at the best frequency of 700 to 800 Hz. Thresholds exceeded 85 dB SPL above 4 kHz.
7. The tracheal system is an important sound route to the ear in some insects [H. Nocke, *J. Comp. Physiol.* 110, 25 (1975)].
8. The 13 to 16 scolopales were visualized in a related species, *Tenodera aridifolia,* using the Masson-Baker stain [D. Young, *Phil. Trans. R. Soc. Ser. B* 256, 401 (1970)]. They were oriented along the ligament so that their distal attachment is to relatively thick cuticle at the edge of the tympanal depression. It is not clear whether the tracheal sac vibration or motion of tympanum itself is transduced.
9. The maximum time of arrival difference between the tympana would be less than 0.5 μsec. Intensity differences at the two tympana due to sound shadowing at 35 kHz are not possible since they are less than 1/50 wavelength apart (2). A system using phase differences (a pressure difference receiver, for instance) could theoretically provide directional information but normally would be effective only at frequencies below the best hearing range of the mantis [A. Michelsen, *Z. Vergl. Physiol.* 71, 102 (1971)].
10. E. Liske and W. J. Davis, *Anim. Behav.* 32, 916 (1984).
11. M. H. Robinson and B. Robinson, *Science* 205, 825 (1979); M. Edmunds, *Entomol. Month Mag.* 111, 53 (1975).
12. K. D. Roeder, *Nerve Cells and Insect Behavior* (Harvard Univ. Press, Cambridge, MA, 1967); L. A. Miller, *J. Comp Physiol.* 131, 113 (1979); A. Moiseff, G. Pollack, R. Hoy, *Proc. Nat. Acad. Sci. U.S.A.* 75, 4052 (1978).
13. We thank A. Bass, R. Capranica, J. Doherty, G. Eickwort, C. Hopkins, H. Howland, and M. Nelson for helpful comments on the manuscript; M. Nelson for drawing the anatomical figures; R. Capranica for loan of equipment; R. Morgan and F. Elia of the Cincinnati Zoo for the Asian mantises; and S. Mancil for typing the manuscript. Supported by an NIH grant to R.R.H.
2 July 1985; accepted 5 November 1985

Infantile Experience with Suckling Odors Determines Adult Sexual Behavior in Male Rats

THOMAS J. FILLION* AND ELLIOTT M. BLASS

Because infant rats learn about odors that elicit suckling, and because certain chemosensory cues that help elicit mating behavior in adults are similar to those that elicit suckling, an experiment was undertaken to assess the influence of suckling-associated odors experienced during infancy on adult sexual behavior. Rat pups lived with and suckled dams whose nipple and vaginal odors were altered with citral, a lemon scent. The rats were weaned and never exposed again, until testing, to citral or females. At about 100 days of age, the males were paired in mating tests with a normal sexually receptive female or with a sexually receptive female that had been treated perivaginally with citral immediately before testing. The males ejaculated readily when paired with citral-treated females but were slow to achieve ejaculation when paired with normal females. These findings implicate an infantile experience as a determinant of adult sexual behavior in a mammal.

THE POSSIBLE EFFECT OF EARLY experience on adult sexual responsiveness has long been a subject of debate. Whereas some birds "learn" in infancy certain features typifying the preferred mate (1), such extreme plasticity has not, to our knowledge, been demonstrated in a mammalian species (2). We report here that adult male Norway rats respond sexually to a chemosensory feature of the sexually receptive female that was associated with suckling behavior in infancy.

Chemoreception is crucial to both suckling and mating in rats. Suckling is prevented by anosmia (3) or nipple lavage (4), and mating is severely impaired by anosmia in sexually inexperienced males (5). Certain sulfur compounds affect the expression of both behaviors (6). In the case of suckling, such chemosignals appear to be acquired, as an artificial odor can be substituted for normal suckling stimuli (7).

Functional equivalence between suckling and sexual chemosignals is directly indicated by recent findings regarding "probing," an olfactorily controlled component (8) of suckling behavior in which the pup repeatedly pushes its snout into the dam before attaching to a nipple. Probing is elicited not only by maternal odors but also by the odors of sexually receptive (estrous) females (9). Moreover, this responsiveness, like responsiveness to maternal suckling odors, reflects particular rearing experiences. Specifically, pups reared with dams whose nipple and vaginal odors—odors that normally elicit probing—were altered by daily painting with citral, a lemon scent, probed normal estrous females only rarely, but readily probed estrous females scented with citral (9). In the study reported here, males reared in this manner until weaning, and then isolated from both females and citral until adulthood, mated more readily with citral-scented than with normal estrous females.

One group of male pups (group C, $n = 39$) was reared with dams whose nipple and vaginal areas were painted with citral (0.03 ml per dam per day; Aldrich) beginning 2 days before parturition and continuing until separation from the dam on day 28. A control group (group S, $n = 24$) was reared with dams painted in the same areas with isotonic saline. A second control group (group CB, $n = 16$) was reared with dams whose backs only were citral-scented. CB rats, therefore, experienced the citral odor in association with the dam, but not as a conjunct of suckling. All males were reared in mixed sex litters.

After weaning, the males were housed in groups in a room free of citral until being tested at 90 to 120 days of age. Each male was then observed in his first postweaning encounter with a female. All testing took place in wire mesh hoops 45 cm across by 30 cm high under dim red illumination during the first half of the animals' dark cycle. The animals were tested two at a time (one male per hoop, hoops 60 cm apart) in videotaped sessions. Each male was allowed 10 minutes alone in the arena before a female was gently lowered into it. All the females were in natural estrus, but half of them had been scented perivaginally with citral (0.015 ml)

Department of Psychology, Johns Hopkins University, Baltimore, MD 21218.

*Present address: Department of Psychology, Yale University, Box 11a, Yale Station, New Haven, CT 06520-7447.