Chapter 8

Chemical Communication in Cockroaches

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Reprinted from: Bell, W. L., 1992. Chemical communication in cockroaches, Pages 133-140, *in* Tested studies for laboratory teaching, Volume 6 (C.A. Goldman, S.E. Andrews, P.L. Hauta, and R. Ketchum, Editors). Proceedings of the 6th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 161 pages.

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Introduction

Species-specific odors are used by a variety of insect species, as well as other animals such as deer, wolves, and rats, to convey information from one individual to another. In fact, throughout the animal kingdom, communication by odors is far more common than communication by vision or sound. Typical information that is transferred by chemicals includes alarm, territory held by an individual, sexual receptivity, and sex identity.

The cockroach is an inexpensive and easy-to-rear laboratory animal that lends itself well to many behavioral and physiological experiments. The objectives of this exercise are to prepare a sex pheromone source of the female American cockroach, *Periplaneta americana*, and to employ the chemical in experiments that demonstrate chemical communication and chemo-orientation. The experiments are directed at college-level students, but would also be appropriate in some secondary school situations. The experiments require approximately 2 hours, but obtaining the sex pheromone requires some preliminary work. For further reading see Barth (1970) and Bell (1981a, 1981b).

Experimental designs for a behavioral bioassay of sex pheromone concentration, procedures for monitoring orientation of male cockroaches to a pheromone source, and some suggestions for observing courtship behavior are presented, along with questions (and answers) and experimental variables.

Experiment 1: Sex Pheromone Bioassay

The following technique can be used to quantitatively assay the concentration of female American cockroach sex pheromone by observing male locomotory behavior.

1. Place five (5) adult male cockroaches into a cage or box approximately 16 cm wide, 25 cm long, and 15 cm high (Figure 8.1). The males should have been maintained on a reversed photocycle, as described in Appendix A. The containers should have clear tops so that males can be observed during a bioassay. Rub a very small quantity of petroleum jelly on the inner sides of the container to prevent cockroaches from walking on the top and sides. Draw a line across the bottom of the cage as shown in Figure 8.1. Provide each cage with food and water (rat lab chow or dry dog food, and water in test tubes capped with wet cotton) and place the cages away from colonies containing females for 10–12 days. Isolation of males from females will lower the olfactory threshold and equalize responses of males to a given dosage of sex pheromone.

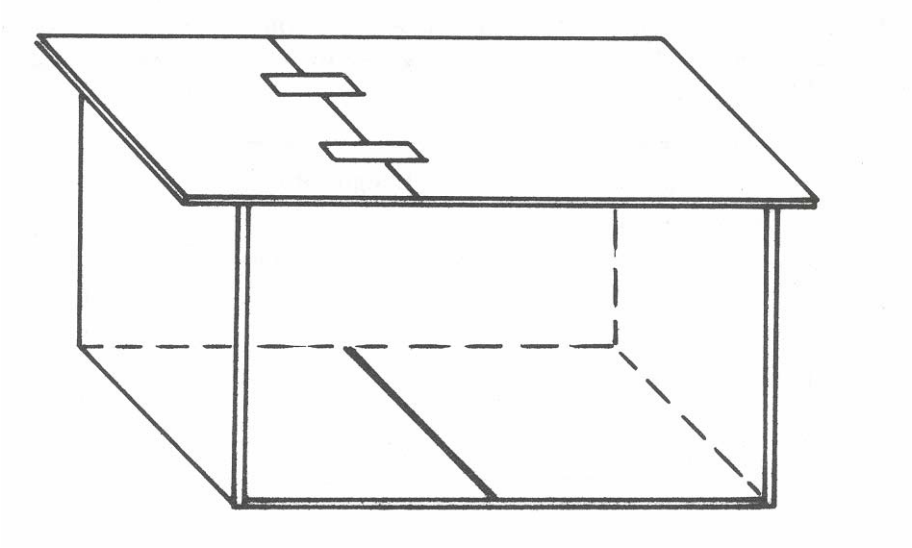

Figure 8.1. Plastic cage with hinged plexiglass top for assaying female sex pheromone. A line is drawn on the bottom of the cage to aid in counting the locomotory activity of males stimulated by sex pheromone.

2. Prepare a female sex pheromone source (see Appendix A for methods).

3. Open the lid of one cage, place a filter paper impregnated with solvent (control) or sex pheromone (experimental treatment) between the top of the cage and the lid so that males cannot contact it. The filter paper can either be one to which 1 ml of sex pheromone extract has been added, or a filter paper from a container of virgin females. Start a stop watch and count the number of times males cross the center line, as a measure of activity. Note that it is the total line crosses of all of the males in a cage that is counted. Record line crosses per minute (activity counts per minute, ACPM) for 2 minutes. Subtract "control ACPM" from "experimental ACPM" to obtain the actual increase in locomotory rate stimulated by the sex pheromone.

4. Line crosses per minute is proportional to the log of sex pheromone concentration (Table 8.1). Of course there will be variation among males and among cages, and so the more cages used per pheromone sample, the more reliable will be the result. Use the assay for a serial dilution of a sex pheromone extract, or simply to find out if sex pheromone is present on a filter paper exposed to virgin females for a certain period of time.

Experimental Variables and Questions

1. Sex pheromone is an odor, and must therefore be perceived through olfactory sensory organs for smell, such as those located in the human nose. What are the olfactory organs of the cockroach? This question can be answered by systematically removing suspected sense organs from male cockroaches (use sharp scissors) and then testing these males in the bioassay with a concentration of pheromone known to elicit a response. *Note:* The olfactory organs are the antennae, the long appendages on the head.

Table 8.1. Line crossings represented as activity counts per minute (ACPM) of males responding to different concentrations of sex pheromone (in μg/ml). Each value is the mean of 10 tests with cages containing five cockroaches.

Pheromone concentration	Length of test	
	1 minute	2 minutes
10^{-2}	55	46
10^{-3}	45	41
10^{-4}	33	26
10^{-5}	27	21
10^{-6}	10	9
10^{-7}	1	0

2. If a bioassay test is continued for a relatively long time, instead of for 2 minutes, do the cockroaches continue to respond to sex pheromone? How long does it take for the response to be curtailed, and how long must the "recovery" period be in order for males to respond again? Is the problem that the olfactory sensory cells stop responding, as in sensory adaptation, or that the central nervous system stops responding to nerve signals from the sensory cells, as in habituation? What effect would the reduction in response to sex pheromone have on male cockroaches which use the sex pheromone to locate a mate? What behavioral strategy might a male use to overcome or circumvent this problem of response reduction? The results will show that the response decreases and then stops after about 10 minutes, and requires a recovery period of about 30 minutes. The "problem" is sensory adaptation.

3. *Do individual males have different response times?* Mark the males used in a bioassay (different colors of fingernail polish or numbered, sticky tape labels), and expose them to a sex pheromone source. Record the time before each individual begins to run around the container. Repeat the test after 1 hour, and record the response times again. Is response time of an individual the same in sequential testing? If so, would fast responders have an advantage in finding mates? What other factors might be involved? *Note:* Individuals vary considerably in their responses.

4. *Do females secrete sex pheromone after they have mated, or only before they have mated?* To answer this question, allow a virgin female to mate, and then keep her in a beaker lined with filter paper. Bioassay the filter paper on days after mating. Wash the beaker each day and use fresh filter paper. If females stop secreting sex pheromone after mating, does secretion resume after the eggs are deposited? For how many egg cases can a female store viable sperm? *Note:* Mating suppresses sex pheromone secretion; sperm can be stored for the production of 2–3 egg cases.

5. *Are cockroach sex pheromones species-specific?* Place male cockroaches of another species in the containers and assay their response to the American cockroach sex pheromone. *Note:* The pheromones are species-specific.

Experiment 2:
Orientation of Males to a Source of Female Sex Pheromone or to a Virgin Female

The bioassay demonstrated that a response of male American cockroaches to perception of female sex pheromone is to run quickly, but more directed behavior must also be involved for a male to actually locate a female that is secreting the chemical signal. Place a sex pheromone source in an experimental arena containing several male cockroaches to observe *how* they locate a pheromone source (or a virgin female). See Appendix A for arena construction.

Experimental Variables and Questions

1. *Do males locate a sex pheromone source by random searching?* Mark off the arena into concentric circles using a black marking pen. Designate the center circle as Zone 1, the next as Zone 2, and so on until the zone at the edge is the last zone. Place a control (solvent) filter paper or a sex pheromone source in the center of the arena, and record the number of times a cockroach enters each zone. Your data will be series of numbers recorded until the cockroach contacts the sex pheromone source; for example, 6 (edge), 5, 6, 5, 4, 3, 2, 1 (contact). Compare the series of numbers to see if more are sequential than are non-sequential. If more runs are sequential than non-sequential, it is probable that random search is not used by the cockroach, but that its orientation is directed by the odor gradient. Are there other characteristics of the orientation response that can be quantified? *Note:* Males initiate their search along the edge of the arena, then switch to random orientation, and finally, within 30 cm of the pheromone source, they exhibit directed orientation.

2. *Do cockroaches use spatial or temporal information processing to decipher an odor gradient?* By now you know that the antennae are the olfactory organs that contain the olfactory sensory cells, and you would probably hypothesize correctly that sensory neurons from the antennal cells converge upon the olfactory lobes in the brain. The cockroach brain could decipher the pheromone gradient either by comparing the input from each antenna (and running in the direction of the antenna that perceives the highest stimulus intensity), or by comparing the combined inputs from both antennae (and running straight if the stimulus intensity increases over time, and turning if the stimulus intensity decreases over time).

   Comparison between antennae is called "tropotaxis," and comparison over time is called "longitudinal klinotaxis." To determine which mechanism a male cockroach uses to decipher the pheromone gradient, compare the time required for males with one or two antennae to contact a sex pheromone source. Your data might consist of two means which can be compared statistically, or your data might show that males with one antenna simply cannot locate a sex pheromone source. Are there characteristics of the orientation of males with one antenna that seem to differ from those with two antennae? Is it possible that males normally use both tropotaxis and klinotaxis? Which mechanism would be most efficient at a long distance or a short distance from an odor source? How do people locate an odor source, such as smoke from a fire? *Note:* Cockroaches with only one antenna can locate a pheromone source, but their orientation time is slower than that of intact cockroaches. The experiment shows that temporal comparisons can be used in deciphering a gradient, but that it is somewhat less efficient than comparisons between the antennae.

Experiment 3:
Sensory Cues Involved in Courtship Behavior

Courtship behavior can be elicited by placing a male cockroach into a cage containing a virgin female. The actions are rather swift, and so students should watch carefully.

Experimental Variables and Questions

1. *What are the sensory cues involved in courtship behavior?* Finding a female is just the first step leading to copulation, and careful observations are required to determine the sensory cues provided by both partners in the interplay that is termed courtship behavior. Use the arena, as described above, but allow a virgin female to run freely in the arena. Actually 2 or 3 females may be needed, because females may be stressed by being introduced into a strange arena. This experiment will promote male courtship behavior, the responses of a female to a male's behavior, and copulation. Attempt to delineate the behavioral sequences involved in courtship: what does the male do and what is the female's response?, and what does the female do and what is the male's response? Are courtship sequences always exactly the same? Are all sensory cues chemical, or are other modalities involved? *Note:* There is considerable variation in sequences of courtship acts, a marked difference from the neat, untrue schemes published in many textbooks for a variety of animal species.

2. *Can courtship behavior of a male be induced by a female-like object in the presence of sex pheromone?* How does a male know that an object is a female? Will males attempt to copulate with any object that smells like sex pheromone? Rats also have sex pheromone. Would a male rat try to copulate with a piece of filter paper containing sex pheromone? *Note:* Male cockroaches will attempt to copulate with objects that are similar in size to female cockroaches.

Literature Cited

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APPENDIX A
Materials and Methods

Preparing a Sex Pheromone Source

1. Check a colony of American cockroaches each day (or as often as possible), watching for newly-emerged adults (white, with wings). Remove these individuals and sex them (see Figure 8.2), placing the males back in the colony and putting the virgin females into a container lined on the bottom with filter paper. For one female, which actually is sufficient for the experiment, a 250-ml beaker will suffice; for five females use a 500-ml beaker, and double the volume for additional groups of five females. Since the objective is to obtain *virgin* females, all will fail if a male or two accidentally joins the group. Note that females are unlikely to mate within 72 hours of molting to an adult.

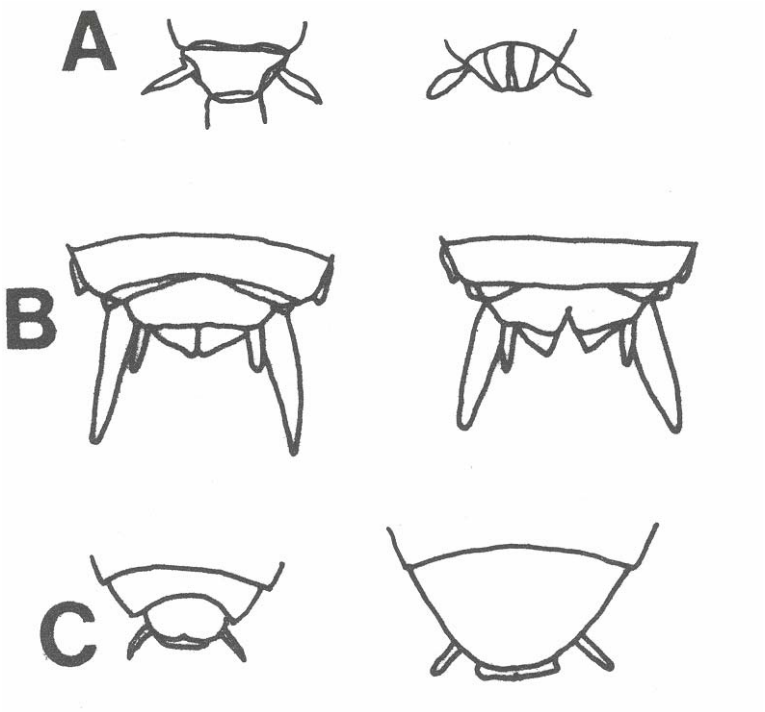

Figure 8.2. Sexing cockroaches: *(A) Periplan eta* adults: male dorsal view *(left)* female ventral view (*right*). (*B*) *Periplaneta* and *Blatta* immatures (both ventral views): male (*left*), female (right). (*C*) Adults of *Blaberus* and *Leucophaea* (both ventral views): male (*left*), female (*right*). Drawings are not to scale.

2. Females begin secreting sex pheromone on about the fifth day after emergence, and peak pheromone production occurs on day 12. The sex pheromone will accumulate on the filter paper such that filters can be replaced every other day from days 5 to 12. To maintain pheromone activity on filter papers, place them in a sealed glass petri dish in the freezer.

3. The instructor should decide if the pheromone is to be extracted and used in a solvent, or if the filter paper itself taken directly from virgin females will be used. The only advantage of extraction is to incorporate chemical procedures into the exercise; *all of the experiments will work just as well with a single filter paper from a container of virgin females as with the extract.*

Pheromone Extraction Procedure

1. If sex pheromone is to be extracted, add as much filter paper as possible to containers holding virgin females. Filter paper strips (3 × 12 cm) are better than discs. Add as many virgin females as possible to the container (50 females will produce a most potent extract).

2. Remove filter paper from the container after about 1 week, fold or roll the paper into the smallest dimensions possible, and insert into the smallest test tube that will hold the paper. Add methylene chloride to the level of the paper and cover tightly. After 2 hours remove the methylene chloride and store in a covered vessel in the refrigerator. Add another aliquot of solvent to the filter paper and repeat the above procedure. Bioassay both aliquots using the methods outlined in the text. If there is no difference in activity between aliquots 1 and 2, combine; if aliquot 2 is substantially less than 1, discard aliquot 2. The goal is to obtain a small volume of highly active sex pheromone extract. The pheromone is active at a concentration of 10^{-4} μg/ml, which is a very dilute solution.

Preparation of Experimental Arena

1. Prepare an arena that students can see into, but from which cockroaches cannot escape. An arena can be constructed from rigid plexiglass, sheet metal, or by tacking the kind of plastic used to insulate windows onto a circle of wood or onto the edges of a table. Circular arenas are better than square ones, because cockroaches tend to hide in corners. The most dramatic results will be obtained with an arena 1.5–2 m in diameter. Regardless of how the arena is constructed, coat the inner sides with a very thin layer of petroleum jelly.

2. For best results, allow the males to reside in the arena at least over night, to reduce stress. Even cockroaches are unlikely to be interested in sex if they are placed into a strange or new environment. Add food and water along the sides of the arena. If possible, dim the lights or perform the experiments under photographic darkroom lighting.

3. Prepare a wire cage or tube from hardware cloth into which will be placed a female cockroach for testing attractancy to males. The reason for the wire cage is to prevent copulation until that aspect of the experiment is desired. If the planned experiments are to include copulation, a tethered female can be used instead.

Cockroach Cultures and Photocycles

At $1.00 per cockroach on the open market, culturing these insects on a small-scale makes sense. American cockroaches can be trapped in the southern United States using food bait in large jars, or a starter colony can be purchased from a supply company. Commercial lab chow is preferable to dog food. Colonies expand quickly, especially if maintained at 75° to 78°F, and in a relatively humid environment. Within 6 months you should have hundreds of immature cockroaches, and in 9 months, many adults should emerge.

For best results the bioassay should be during the dark phase of the males' photocycle performed under dim or red (photographic darkroom) lights. To maintain cockroaches on a reversed photocycle, place the cages of males in a closet, incubator, or box, and put a light bulb on a timer so that the dark period for the cockroaches is approximately 7 a.m. to 7 p.m.

Special Materials

1. Disposable mouse cages or plastic shoe boxes are ideal for bioassay containers. Cover with plexiglass lids or pieces of glass.

2. Mechanical counters are very handy for counting the number of times cockroaches cross the line.

3. If video equipment is available, tape the courtship or the orientation of males searching for females to allow students to re-examine these rather fast movements in a quantitative manner.