Chapter 10

Olfaction and Chemical Communication

James Traniello

Boston University, Department of Biology
5 Cummington Street, Boston, MA 02215
(617) 353-2832
FAX: (617) 353-6340
EMAIL:jft@bio.bu.edu

James Traniello, Professor of Biology at Boston University, specializes in the behavioral ecology and sociobiology of insects. He received his Ph.D. from Harvard University. Professor Traniello has received several research grants and outstanding teaching awards. Along with Dr. Elizabeth Godrick, Professor Traniello received NSFUSE-9155953 to develop this exercise.

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Contents

Introduction

Goals

- The exercise emphasizes the way in which science is carried out; experimentation takes places over 2-3 weeks and directly relates to and prepares students for modern research-oriented upper division biology courses.
- The theme of this module ties in closely with the behavioral portion of the lecture component of the course.
- This Olfaction Module (1) exposes students to basic questions in biology 2) gives direct experience with scientific methods and approaches required to deal with those questions.

Studies of animal communication provide students with an excellent opportunity to design and conduct simple, elegant analysis of social behavior. Through the experimental separation of sources of social stimuli, the development of appropriate bioassays and the recording of behavioral data and subsequent statistical analysis, students are not only able to gain hands-on experience but also are enabled to understand levels of inference and types of proof used in science. In the present study, for example, students are unable to see the pheromone molecules which mediate alarm and recruitment, but through experimentation they can gather data to provide circumstantial evidence of the source of the pheromones which regulate these social interactions.

Techniques such as microdissection and extract preparation are also developed through this laboratory exercise. Finally, there is excellent original literature available to illustrate the "pioneer" research into the field of communication, as well as various types of popular articles. In essence, students will in this exercise duplicate the original methods used by Edward O. Wilson to determine the origin and effects of pheromones. We are also able to bridge chemistry to allow students to understand the relationship between molecular structure and function, thus emphasizing an interdisciplinary approach. Termites and ants are chosen because they provide a whole non-endangered organismal model to incite student interest and are easy to manipulate and maintain.

Experimental Design

If desired, instructor can plan this module for a 3 week time period

Week 1

Students can learn skills in observing social behavior by working with displays of whole colonies of ants and termites. They can become familiar with colony organization and inter-colony relationships including food sharing and grooming behavior. During this first session, students can also observe prepared

histological sections of insect anatomy to become familiar with pheromone sources. Original and secondary literature sources can be reviewed with attention to student reporting or research.

Comeback Time: students may complete any aspect of unfinished laboratory and/or plan future experiments.

Week 2

Students will anesthetize and decapitate *Camponotus*, *Pogonomyrmex*, or *Formica* ants in order to remove their mandibles and the attached pheromone secreting mandibular gland. Students can crush gaping, antennae waving, fast running, aggression, biting, avoidance, touching.

Students can then determine degree of specificity of the alarm response of workers by observing the effect of other chemicals such as ethanol, citronella, glyceral, methyl heptanone, and undecane, and subsequently account for differences.

Finally, students can study trail communication by removing the sting with attached poison and Dufour's glands from anesthetized ants. (students with bee sting allergies will not handle ants.) Trails of each extract can be drawn and ant behavior from different colonies can be recorded and described.

Comeback Time: to complete unfinished work; written plans of third week's bioassay for termite trail pheromones can be discussed with mentor.

Week 3

Based on experience and readings of former weeks, students will devise a bioassay and procedure to bioassay and identify the source of trail pheromone in the common social insect the termite, *Reticulitermes flavipes*. The last hour can be devoted to discussion of individual results.

Comeback Time: written report of results will be completed and submitted.

Materials

Ants
Forceps, Dumont No.5
Formic Acid
1-octanol
Decyl Acetate
Hamilton Syringes
Insect Pins
Aluminum Foil
Saran Wrap
Screen Charge for Logo (for T-shirts used in human odor communication exercise; optional)
Hamilton Syringes
Petri Dish, 150x25
Microflex Vials, 0.3
Capillary Tubes
Reticle
Fluon AD1

Notes For Instructors

General Tips

1. Always use fresh ants, that is, ants that have been recently isolated from their nests. This is particularly important in end-of-the-week labs, because ants have been probed and hassled all week. There is simply no way of listing what ants will be found, because this will depend on where one is in the US/Canada. People will have to adapt the protocols to their abilities/location.
2. Don't overfeed colonies; they'll get saited and lazy. This is particularly true for the demonstration of trail communication: well-fed ants won't respond to food
3. If the microsyringes don't work well because they are plugged, and you can't fix them, use a micropippette.

Determination of the Origin of the Trail Pheromone in Reticulitermes Termites

1. Obtain a termite (Reticulitermes flavipes) from a stock colony. Termites, unlike ants, have no sting with associated glands that produce trail substances. Yet the foraging in all species studied to date is coordinated by chemical trails.
2. With forceps, decapitate the termite and separate the thorax from the abdomen. Make sure the legs have been removed. Keep the orientation on the abdomen so that you know the dorsal side from the ventral. Eviscerate the abdomen so that all the viscera come out. You can do this by holding the abdomen (gaster) with one set of forceps and introducing the tip of another forceps onto the cut ends of the gaster and pulling out the viscera. Pull carefully on the pleural membrane to remove the dorsal side. Retain the ventral segments of the cuticle as the glands are located here. Place them on a droplet of saline in the depression of a wax bottom dissecting dish. The sternal gland is located between the fourth and fifth abdominal segments. It looks like one of the ventral ganglia but is more translucent, and does not have any nerve cord associated with it. NOTE: If you cannot separate the sternal gland from the cuticle, drop the entire preparation into the V-shaped extraction vials.
3. With the blunt end of a glass capillary tube, grind the preparation in 10 µl hexane. With a pencil, draw your "V" trails on a paper. With a microsyringe, take up the extract and dispense it (trace) over one side of the "V." The other side, which will have traced over it only 10 µl hexane, represents the control.
4. Place the test paper into the termite arena. Place several (20-30) termites on the paper at the junction of the control and experimental trails. NOTE: The above protocol is to be used as the correct "solution" to the problem presented to the class, which is to determine if trail communication occurs in termites, and if so, to find the origin of the trail pheromone. You should present this to the class as "Do subterranean termites use trail pheromones?"

You could stimulate thinking about the problem with the following questions:

a. Does the biology of subterranean termites favor the evolution of trail communication? (Colonies are very large [potentially millions], and nest in the soil in darkness; they feed on large food sources such as stumps and fallen trees, which favor cooperative foraging).
b. What simple experiments or observations could be made to determine if trail communication occurs? (Place [dump] about 50-100 termites in a large petri dish [15cm diameter]; soon after, you will see trails form. Also, you could have small colonies in sand nests in 15cm petri dishes, kept overnight without food, which can be offered during lab. You will see recruitment occur.)

Student Outline

Pre-lab Questions: Olfaction and Chemical Communication Laboratory Module

1. What types of organic compounds might serve as alarm pheromones?
2. What is an exocrine gland? Which exocrine glands produce alarm and trail pheromones in ants?
3. What are the differences and similarities in the structure of odor receptors in insects and vertebrates?
4. Describe how you would bioassay a chemical for alarm effects in an insect.
5. What is Q? What is K? How are they used to study behavioral responses to pheromones?

Olfaction And Chemical Communication

Introduction

Our perception of the world around us is biased by our senses. We humans process information in our environment through vision, hearing, touch, and smell. The information we detect is used in orientation and feeding, to name two important contexts, and also in communicating with other individuals. Human language, which includes both verbal and non-verbal signals, is the most frequently used mode of communicating. Although our olfactory sense today seems to play a minor role in human communication, it is likely that during early human history olfaction was more important. The ancestral role of olfaction in human communication is suggested by comparisons with other primate species. Some physicians believe that odors serve an important function in human psychosexual development, and there have been many new insights into human odor communication during the past twenty years. But there are still relatively few studies of chemical communications in humans.

In contrast to humans, there are many excellent studies demonstrating an extreme reliance on odor for communication in other animals. In fact, chemical signaling is the oldest and most widespread form of communication, and has been described in all phyla with the exception of birds. Some animals perceive nothing but a chemical world, and all of their behavior is regulated by a relatively small number of substances. One group of animals that relies heavily on chemical communication signals, or *pheromones*, is insects. Research into insect chemical communication has greatly increased our understanding of animal behavior, and a large number of pheromones have been chemically identified. Some of these chemicals are used to control pest insect populations in an ecologically sound way, with minimal environmental impact because the chemicals used are specific to a pest species and are not toxic.

Insects that live in colonies have provided model systems to study chemical communication. Social insects (all ants, all termites, some bees and wasps) may exist in large groups - often tens of thousands and sometimes millions - and perform tasks as groups through cooperation among individuals. This cooperative group behavior arises from the coordination of the actions of individuals, and this coordination is achieved by chemicals that regulate social behaviors. For example, in tropical rainforests in the New World, leaf-cutter ant colonies - which may contain two million individual ants - nest in the soil in a series of underground chambers. Scout ants search for suitable vegetation to cut and collect to rear fungus to eat; a single tree may be the source of much leaf fodder, but it would be inefficient for only a single ant to exploit such a rich food source. The scout ants will, however, lay chemical trails to guide their nestmates to the tree, and all will cooperatively cut and harvest leaves. The chemical trail, or trail pheromone, stimulates ants to leave the nest and orient to the food. The trail pheromone in this case is methyl 4-methylpyrrole-2-carboxylate ($C_7H_8NO_2$), which is produced in the poison gland of worker ants and is discharged on the

ground through their sting. This trail pheromone has incredible biological activity: only 1 milligram of the pheromone could lead leaf-cutter ants around the circumference of the earth three times.

Another context of chemical communication involves *alarm signaling*. When a colony is disturbed, workers emit pheromones that evoke alarm behavior in nestmates. Sometimes the alarm behavior shown by workers is aggressive, and they will begin to attack. On the other hand, some alarm pheromones induce a "panic" response, and individuals that detect the signal will abscond. Different species may show different alarm behavior, even to the same pheromone.

There are few aspects of social insect behavior that are not mediated by olfactory communication, and the chemical senses of social insects are extremely well developed. Consequentially, many paradigms in olfactory communication have been established using different species of social insects. Two groups - the ants (Order Hymenoptera, Family Formicidae) and the termites (Order Isoptera) are particularly well-studied. In contrast, chemical communication in higher vertebrates, like mammals, is more difficult to study because of the complexity of vertebrate social behavior and the difficulty in analyzing their behavior experimentally in the field and laboratory.

In this series of laboratories we will study olfactory communication. To begin, we will examine olfaction in humans by looking at the relationship between molecular structure and odor perception and human response to different chemicals. Then chemical signaling will be studied in ants and termites, using an experimental approach. These latter exercises will demonstrate the role of the physical properties of chemicals in the evolution of pheromone communication and the measurement of factors important to the analysis of the biological properties of chemicals.

Human Olfaction

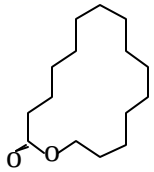
In humans and in all animals, odors are detected by *chemoreceptors*, nerve cells that are specialized to perceive chemicals. A chemoreceptor is a neuron that has its cell body in the epithelium of the nose and its dendrites projected into the mucous layer of the epithelium surface. Chemoreceptor cells depolarize when odorant molecules bind to sites on the dendrite, and a nerve impulse occurs. Human chemoreceptor cells can detect small changes in molecular structure. For example, changing the position of a group relative to a double bond can produce very different odor perception. The aldehyde citral-A (geranial) smells like lemon, and the *trans* isomer citral-B smells like rose.

Humans are able to distinguish thousands of different odors. But do any chemicals play a role in human communication? The perception of exaltolide is one example often cited to provide evidence of human pheromones.

In the first investigation, you'll collect and evaluate data on the perception of exaltolide, and discuss the implications of your data.

Detection of exaltolide

Chemically, exaltolide is the hydroxy lactone of decanoic acid, or pentadecalactone. Its structure is shown below.



Samples of exaltolide and other compounds stored in small glass vials marked C_1, C_2, and C_3 are available from your instructor. Each working group of students should receive one set of vials. Open the cap to each vial and cautiously take a whiff of the chemical that has been placed within.

What do you smell? Obtain an odor perception data sheet from your instructor and answer the questions on the sheet. Note on the sheet your subjective assessment of what you have perceived in each vial. (That is, "I don't smell anything," or "I detect the odor of orange rind.") Use the following semi-quantitative scale to rate your response. For example:

0 1 2 3 4 5

no odor *weak odor* *strong odor*

Note: You do not need to write your name on this sheet, but do turn it in to your instructor at the end of the exercise.

Part of the section on human olfaction in this lab module will involve designing experiments to measure human response to odors. These are the well-known "T-shirt" studies. This project will be done outside of the lab and will be discussed by your instructor.

Chemical Communication in Social Insects

Now we take a big leap from humans to insects.

As we said earlier, many social insects rely heavily on pheromones to communicate. Each insect in a colony has the anatomy and physiology required to send signals as well as the sensory organs and nuerophysiological responses needed to detect and interpret them. To study chemical communication in social insects, lets first find the organs that produce pheromones. After you understand where the glands are found, and what they look like by viewing diagrams and photographs of histological sections, you will then try to find them yourself in a live animal. All you need is a good microscope, fine-tipped forceps, steady hands and good hand/eye coordination, *and patience!*

Pheromone sources

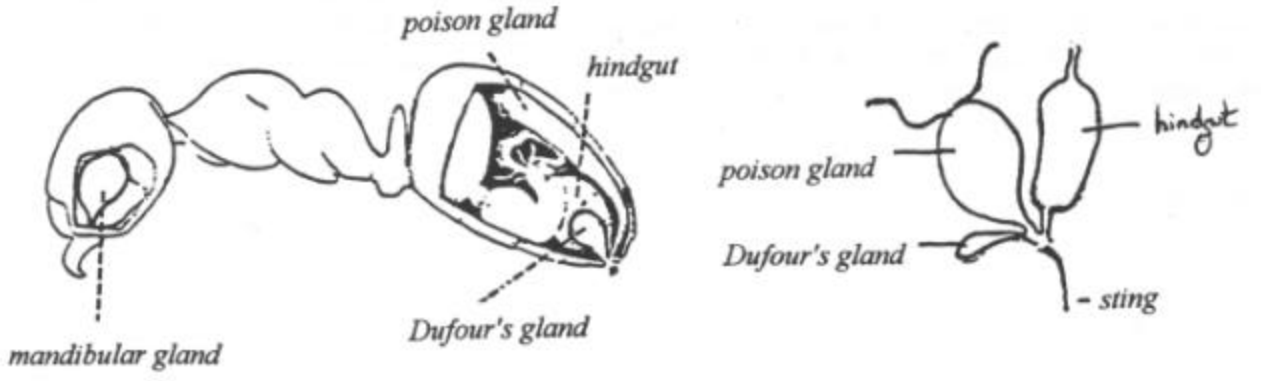
Pheromones are synthesized and stored in exocrine glands, which vary in position and number in different species. A worker ant, for example, is a sterile female. In some species, workers have a sting that they use to defend themselves (the Pogonomyrmex have one - be careful handling them!). The sting is an ovipositor (egg-laying guide) that has evolved into a defensive weapon. Some pheromone-producing glands are associated with the sting; others are found near the mandible (another potential defensive weapon- these are jaws that are used to bite) or occur between body segments. These glands often secrete alarm pheromones which are chemicals that cause individuals to become alarmed and aggressive, or abscond.

Examine the figures of the exocrine gland anatomy of different social insect species to get an idea of the location and structure of pheromone producing glands.

Exocrine gland dissections

Using Dumont No. 5 fine-tipped forceps, dissect out the Dufour's and poison gland from the chilled worker *ants (Lasius sp., Myrmica sp., Aphaenogaster, Pogonomyrmex badius,* e.g.). For each dissection, grip either the sting (*Pogonomyrmex, Myrmica*) or the tip of the gaster (the ant equivalent of the abdomen; *Lasius, Camponotus, Formica*) with the forceps while holding the remainder of the gaster

with a second pair of forceps; gently pull. Place the dissected glands in a small pool of saline in a wax-bottomed dissecting dish. If the dissection has been performed correctly, you should be able to see the poison gland, the Dufour's gland, and the hindgut, which will appear as follows:



To remove the mandibular gland, decapitate a cold-anesthetized ant (any species) and remove a mandible ("jaw") by grasping it with a forcep and move it in the opposite direction of its normal movement (the left mandible should be moved clockwise, the right one counter-clockwise). This procedure isn't easy, so don't get frustrated. Examine the mandible and attached structures in a pool of saline as before. You should see muscle tissue and a sac-like reservoir, which is the gland itself. Practice the dissection of exocrine glands until you are confident you can remove and recognize each gland.

Note: Ants is the subfamily Myrmicinae (*Aphaenogaster, Pogonomyrmex, Myrmica, Tetramorium*, e.g.) have two petiole nodes which distinguish them from ants in the subfamily Formicinae (*Camponotus, Lasius, Formica, Acanthomyops*, e.g.) having one petiole. The petiole is a body segment found between the thorax and gaster (abdomen).

Pheromone perception

The sense organs that detect pheromones are the paired antennae on the insect's head. Each antenna is comprised of a ball-and-socket type joint on the head, a long scape, and a series of approximately 10 segments making up the funiculus.

a) Examine a prepared slide of an antenna of an ant. How does the scape differ from the funicular segments?
b) Observe ants in a plastic observation nest. How are the antennae used? In which social behaviors are they involved?
c) Why are the antennae paired? How can such a pair of sense organs be used to sense odors?
d) Compare insect antennae to the nostrils of a mammal (humans are a good example). How does the placement of the olfactory receptors differ in the scale of the size and location of these sense organs?
e) Examine the drawing comparing the olfactory sense organs of an insect and a vertebrate. What are the differences and the similarities?

Pheromone emission

NOTE: Any student with a bee-sting allergy is NOT to handle *Pogonomyrmex* ants.

Obtain a small worker group of the ants *Acanthomyops, Camponotus,* and *Pogonomyrmex*. Grasp one with a pair of forceps. Cautiously try to detect any odors that are emitted from the worker you are holding. Compare the odorants from these species. Are the odors similar or different?

Behavioral Response to Pheromones

There are a number of ways the behavioral response to a pheramone can be measured. All involve the use of a *bioassay*, that is, a test in which the behavior of a live animal is quantified to determine the effect of a stimulus such as a chemical. So, the response of the presence of one animal near another can be bioassayed, as can the response to the contents of an exocrine gland, a particular fraction of gland extract on a HPLC (high pressure liquid chromatography) trace, or a synthesized chemical obtained from a supply house. The first step in analyzing a response to a pheromone, and the simplest procedure, is to describe the response of individuals to freshly-dissected exocrine gland contents. In effect, you try to duplicate the natural response using gland secretions.

a. Describe an alarm response under semi-natural conditions.

Alarm behavior occurs when an individual is disturbed by a predator or a collapse of a portion of the nest. Once alarmed, others may also become alarmed. So, an alarm response can be propagated through a colony.

Obtain an artificial nest of ants. Being very careful not to disturb the ants by vibration, or breathing, use an applicator stick as a probe placing it through one of the holes drilled in the wall of the container. With the applicator stick, poke one ant, or add an ant of a different species. Describe what happens.

Now you need to quantify the behavioral response you just observed. To do this, make a list of the behaviors you see and record their frequency. Here is a list of behaviors to help you:

Behavior

1. Avoidance (ants move away from a stimulus)
2. Antenna waving (lift antennae and move them toward stimulus)
3. Mandibular gaping (open mandibles, as if to bite)
4. Biting and attack
5. Fast running (agitated movement through the nest)

To qualify the response you see, you could rate the response as:
"0" - none of the above behaviors observed
"1" - avoidance and/or antennal waving only
"2" - open mandibles, some agitated movement
"3" - biting, fast running

In this scheme, ranking the response from 0-3 lets you quantify weak to strong alarm responses. For example, in 5 replicates, you might get scores of 0, 1, 1, 0, 1 when you test one species and scores of 2, 3, 3, 3, 2 when you test another. You could calculate the average response to compare the two species' response (in this case it would be 0.6 vs. 2.6).

b. Induce an alarm response with exocrine gland contents.

Here's the procedure, step by step:

1. Obtain a small ant colony and ant worker from a stock colony. Any species can be used.

2. Anesthetize an ant by placing it in the freezer for about 2 minutes. Decapitate the ant and remove a mandible by grasping it with a fine-tipped forcep and moving it in its opposite direction of movement. (That is, the left mandible should be moved clockwise, the right mandible counterclockwise).

NOTE: Be extremely careful not to damage the forcep tips!!

3. Examine the mandible and attached structures in a droplet of saline on a wax bottom dish under the dissecting microscope. Attached to the mandible you should see muscle tissue and, if your dissection was successful, the mandibular gland which will appear as a sac-like reservoir.
4. Remove the mandibular gland with your forceps and crush it on the tip of an applicator stick. If you cannot dissect the gland itself, crush the head on the tip of an applicator stick. Quickly place the gland (or head) crush 2-3 cm away from workers in your colony. Be careful not to create any disturbance by touching workers or breathing on the nest. Do you detect any odor?
5. Record the response of the workers in the colony, (quantify the response) then withdraw the stick.
6. Wait until the colony is calm and place a clean applicator stick in the same position in the nest as your gland crush and record the response. Why do you perform this later experiment?
7. Allow 5 minutes to elapse and place the applicator stick with the gland crush in the colony again and record the response of workers. Is there any difference? Why?
8. Repeat the same procedure using a dissected poison gland and Dufour's gland (see section 2 for dissection procedure). Tabulate your results after you complete all your bioassays. Compare results between and within species. Also, test the response of ants to an exocrine gland crush from a different species (i.e. test the response of *Lasius* workers to a *Myrmica* or *Aphaenogaster* poison gland crush). Are the alarm responses always the same in different species? You could set up a table to summarize your data, like this: (see section on previous page for measurement of responses)

Table 1. Average response of two ant species to different gland extracts. Response (recorded on a scale of 0-3) is given as the average of 5 replicates on each species.

Gland Tested	Species A	Species B
Poison gland from species B	0.26	3.0
Dufour's gland from species B	0.31	2.4
Control (applicator stick only)	0.0	0.0

Tabulating your data in this way will let you determine if the chemicals in the glands are species-specific, that is, elicit a response in only the species that the glands were taken from. The above table suggests that the pheromones of species B are species-specific; the response of species A was very low in comparison with that of species B.

Do you think that alarm pheromones are species-specific in animals in general? Why or why not?

Measuring the chemical and behavioral properties of alarm pheromones.

Imagine that you have a bottle of dimethyl sulfide, a liquid that has an odor like that of rotten eggs, or a bottle of mixed thioacetates (the defensive secretion of a skunk). At one end of an empty room you open the bottle. The air in the room is completely still. At the opposite side of the room you try to detect the odor of the substance in the bottle. At first you perceive no odor, but over time the odor becomes stronger and stronger, until.....wow! what a stink!.

Chemicals like dimethyl sulfide and thioacetates are *volatile*; they evaporate and molecules diffuse into the air. Over time, the concentration of these molecules becomes greater, until they finally reach a

concentration that is detectable by your nose. If you were to try to sense the odorant at 1, 2, 4, and 8 meters from the bottle, you would detect a *concentration gradient* of molecules. That is, at 8 meters, the odor would be slight, and somewhat stronger at 4 meters. At 2 meters it would be strong and at 1 meter it might be unbearable. Certainly such a concentration gradient of noxious molecules would affect your behavior. If you detected the odor of a skunk at a distance, you would probably move to avoid it.

Pheromones, in particular alarm pheromones, are also volatile substances that when emitted from an exocrine gland diffuse into the air and create a concentration gradient where there are areas of high and low concentrations of molecules. The highest concentration of molecules will be at the source (an ant's mandible or the tip of the gaster); a 3-dimensional "odor sphere" will form as the pheromone evaporates and diffuses. The periphery of the odor sphere will have the lowest concentration of molecules.

If the concentration of pheromone molecules within this odor sphere created by pheromone diffusion is above the threshold number of molecules needed to stimulate a behavioral response, it is described as the active space of the pheromone. You can actually measure the physical properties of pheromones that contribute to the structure of the active space, and the number of molecules required to elicit a response.

This is what you'll need to do next.

The emission rate of a pheromone, denoted by *Q*, tells you the concentration of molecules in the air. The value of *Q* will depend upon a number of physical properties such as its molecular weight and density. In this part of the lab you will determine what kinds of chemicals have properties that would allow them to serve as alarm signals. The substances you will test are listed below. Each is from a class of organic compounds known to serve as an alarm pheromone.

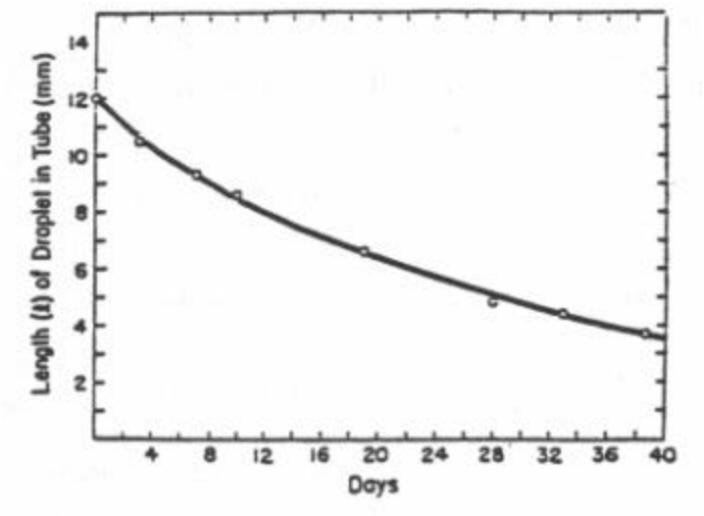
a. Emission rate (Q)

Chemical	**Formula**	**Class**
methyl heptanol	$CH_3(CH_2)_3COH(CH_3)CH_2CH_3$	alcohol
ethanol	CH_3CH_2OH	alcohol
methyl heptanone	$(CH_3)_2C{=}CHCH_2CH_2COCH_3$	ketone
hexane	$CH_3(CH_2)_4CH_3$	hydrocarbon (alkane)
undecane	$CH_3(CH_2)_9CH_3$	hydrocarbon (alkane)
formic acid	HCO_2H	carboxylic acid
decyl acetate	$CH_3(CH_2)_8CO_2(CH_2)_9CH_3$	ester
hexanal	$C_6H_{12}O$	aldehyde
citronellal	$H_2C{=}C(CH_3)(CH_2)_3CH(CH_3)CH_2CHO$	terpenoid

Week 1

First you need to prepare a sample of the substance to be used to measure emission rate. This sample will later be used in behavioral bioassays. For each substance, place roughly 3 µl (use a 10 µl Hamilton 701N microsyringe) in a capillary tube (sealed at one end; 1.0 mm inner diameter). Do this under the hood. Measured from meniscus to meniscus, the droplet in the capillary tube should be about 3-4 mm in length, as seen below:

Leave the tubes at constant temperature (25°C). Over the course of the next week, at approximately one-day intervals (± 2-4 hours), record the length of the droplet of each substance in each capillary tube and plot the change in droplet size (Δl) as a function of time. The plot of $\Delta 1$ will look like this:



Here are the comeback time exercises for weeks 1-2:

1. Compare the Δl values for the 11 substances. What do these comparisons tell you?
2. Using the CRC Handbook of Chemistry and Physics to look up the molecular weight and specific gravity (density) of one or two substances. Tabulate this information. The molecular weights and specific gravities for all the pheromones in this study are listed in the Appendix, but we want you to have an idea of where you would find such information, so that's why we direct you to the CRC Handbook first.
3. Determine the slope of the curve during days 1-3. Plot the slope values for each substance as a function of the molecular weight of the substance (obtained from the table you prepared under step 2 above).
4. What can you conclude about the volatility of the substances?

Week 2

Now that you have prepared your test source for each chemical, you can calculate Q, the emission rate from the following equation:

$$Q = \frac{\Delta l \times \pi\,(d/2)^2 \times 10^{-3} \times \text{density} \times 6.02 \times 10^{23}\ \text{molecules/sec.}}{\text{molecular weight}}$$

where Δl is the rate of decrease in droplet length ,measured in mm/sec.

Use the last two length readings to obtain Δl ; d is the inner diameter of the capillary tube (1.0 mm) Essentially, Q tells you how many molecules of chemical are diffusing from the open end of your capillary tube.

After you calculate the value of Q for each substance, you are ready to determine the behavioral effect of the chemicals. Use the same behavioral scale developed in part 5.1 to quantify the response for two species of ants to each of these substances. When you do this, you will be able to calculate K, the behavioral threshold.

b. Behavioral threshold calculation (K)

The behavioral threshold is the concentration at which the chemical elicits a response in an animal. The behavioral threshold, which is abbreviated as *K*, is calculated to determine if some substances are more effective than others at causing alarm behavior. The value of K tells you if the chemical receptors that respond to substances are more sensitive to one chemical than another. To calculate *K*, use the following formula:

$$K = \frac{Q}{2D\pi r} \times erfc\left(\frac{r}{\sqrt{4Dt}}\right)$$

where: *Q* is the emission rate
D is the diffusion coefficient of the substance
r is the distance, in cm, from the tip of the capillary tube to the receptor (the antenna)
t is the time, in sec., to the onset of the alarm response
erfc is the complementary error function estimated from the figure in the Appendix.

To conduct your behavioral tests, use groups of ants that are in plastic boxes with small holes drilled in the sides. These holes serve as ports for your capillary tubes; insert the tube, measure the distance (r), then with a stopwatch time the interval to the response by the ants to the diffusing pheromone. The response may be as mild as moving the antennae to a quick aggressive response, depending upon the chemical and the species tested. Test at least two different ant species. Tabulate your results. Be sure to first use a control. (What should the control be?)

Now calculate *K* for at least two of the following species and substances:

(Note: different groups of students should choose different pheromones and species; you can then share results to make comparisons).

Species	**Pheromones**	**Diffusion Coefficient (D)**
Lasius	formic acid	0.18
Pogonomyrmex	methyl heptanone	0.06
Myrmica	undecane	0.06
Camponotus		
Formica		
Acanthomyops		
Aphaenogaster		
Tetramorium		

(You may not have all these species available)

Think about and answer the following questions:

1. How do the K values vary for the substances? Are all substances equally effective at eliciting a response? Did workers of some species not respond at all?
2. Based on your answer to question 1, do you think that alarm pheromones are species-specific? Explain your answer.
3. Do you think the chemical identity of alarm pheromones used by a species would give you useful information for studies of systematics?

Week 3 Trail Communication

In addition to communicating alarm, many species of social insects also communicate information about the presence and location of new food sources. Because each forager is small relative to the size of the food items they may collect, it is sometimes more efficient for workers to cooperate during foraging and retrieve

food sources as a group. Scout workers that explore a colony's feeding area find new food and communicate information about it's location to other colony members in the nest. Other foragers are alerted by a trail pheromone discharged on the ground between the food source and the nest and orient along a chemical trail to get to the food. After they feed, they may themselves reinforce the trail through pheromone deposition. You can observe this type of recruitment communication in the laboratory demonstration of ant trail pheromones.

1. Chemical recruitment communication in ants

Offer food (1ml of a 50:50 mixture of honey and water) about 30cm from a nest of ants (*Lasius, Myrmica*, etc.) [just don't use *Pogonomyrmex*-they are seed eaters!].) Describe the behavior you see (i.e. plot the number of ants at the food source over time and the number of trail-laying ants (can you see the difference in posture?)

After the honey water is depleted, foraging seems to "shut off." Why?

2. Experimental analysis of trail communication in termites.

Termites, like ants, communicate information about the location of food sources by using trail pheromones. Your instructor will arrange a demonstration of trail communication in termites (*Reticulitermes flavipes*) for you.

Create a series of experiments that will allow you to determine if trail communication occurs, and, if so, what is the source of the trail pheromone used in *Reticulitermes*. What will you use for a bioassay? How will you record and analyze data?

Odor Cues Used in Gender and Individual Discrimination

Many species of mammals, including non-human primates, use pheromones to repel rivals, mark territories, and signal sexual receptivity. As mammals, humans also have an array of organs and associated structures that would seem to imply that odor cues play some role in social interactions. For example, apocrine glands beneath the arms do not produce only sweat, and these glands have their secretions spread over tufts of hair. Yet such exocrine glands are usually described as "non-functional" and the role that smell plays in human non-verbal communication has been considered to be minimal.

Design a study to determine the role of odor in humans. One relatively simple and interesting experiment would be to determine if individuals can discriminate the odors of same-sex and different-sex individuals (i.e., gender can be recognized through odor perception). In your design methods, data analysis consider the following.

1. How can odor samples be collected? What is your bioassay of their perception?
2. What factors may influence individual odor? How can you control for their influences?
3. What factors may influence odor perception? How can your experiment be designed to allow you to isolate the influence of these factors? Working together in your lab group within your lab section, write up your experimental design.

Odor Perception Data Sheet

Your group has three vials (C_1, C_2, C_3) that are samples for you to assess olfactorily. For each sample, use the rating scale in your lab manual and make any subjective notes on what you perceive.

Sample C_1

Rating: _____

Notes:

Sample C_2

Rating: _____

Notes:

Sample C_3

Rating: _____

Notes:

Answer the following questions. Your answers will be used to compile data on the response to the odorants in the vials. You do not need to give your name.

1. Age:
2. Sex:
3. Ethnic origin:
4. Hours of exercise per week:
5. Do you eat meat? yes____ no____
6. Do you have a preference for spicy foods? yes____ no____

For males only:

Weight:

In the past year, did you steadily date one person? yes ____ no ____

For females only:

Weight:

Time since last ovulation (estimate) _____days

Human Olfaction

Age: _____

Sex: Male_____ Female_____

T shirt #	Odor			
1	Male	Female	Pleasant	Unpleasant
2	Male	Female	Pleasant	Unpleasant
3	Male	Female	Pleasant	Unpleasant
4	Male	Female	Pleasant	Unpleasant
5	Male	Female	Pleasant	Unpleasant
6	Male	Female	Pleasant	Unpleasant

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We wish to thank Mary Lesniak and her staff for gathering and culturing the insects used in these experiments and Dr. Elizabeth Godrick and Marie Mota for the preparation of this document.

APPENDIX A
Calculations

Sample Calculation of Q

On day 1, at time 0, the length of a pheromone droplet in a capillary tube, is 8mm. On day 4, droplet length decreased to 6mm due to evaporation at the open end of the tube. Thus the rate of evaporation, or Δl, was:

$$= \frac{2 \text{ mm}}{4 \text{ days}} \quad \text{(which we want to convert to mm/sec)}$$

$$= \frac{2 \text{ mm}}{96 \text{ hrs.}}$$

$$= \frac{2 \text{ mm}}{3.46 \times 10^{4} \text{ mm}} = 5.8 \times 10^{-6} \text{ mm/sec.}$$

In this example, a substance with a specific gravity (density) of 1.0 and a molecular weight of 100 is tested, and the inner diameter of the capillary tube is 1.0 mm.

$$Q = \frac{\Delta l \times \pi \, (d/2)^2 \times 10^{-3} \times \text{density} \times 6.02 \times 10^{23} \text{ molecules/sec.}}{\text{molecular weight}}$$

$$= \frac{5.8 \times 10^{-6} \times (3.14) \times (1/2)^2 \times 10^{-3} \times 1.0 \times 6.03 \times 10^{23}}{100}$$

$$= 2.74 \times 10^{15} \text{ molecules/sec.}$$

Sample Calculation of K

$$K = \frac{Q}{2D\pi r} \times erfc\left(\frac{r}{\sqrt{4Dt}}\right)$$

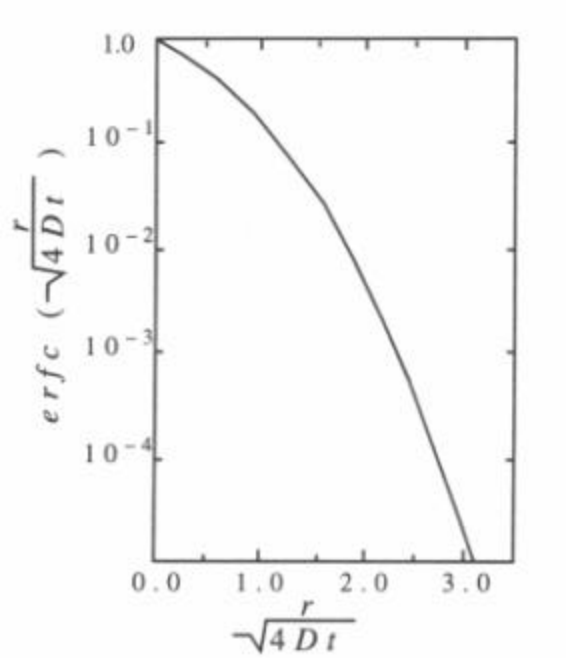
Therefore, we need to know Q, D (the diffusion coefficient of the pheromone), r (the distance of the pheromone source to the responding animal), and t (the response time).

Assume the pheromone is undecane, which has a diffusion coefficient of 0.06. And your r value is 2 cm, and t is 3 seconds. You also need to know the complementary error function.

First, calculate $\frac{r}{\sqrt{4Dt}}$

$$\frac{r}{\sqrt{4Dt}} = \frac{2}{4\,(0.06)(3)} = \frac{2}{0.85} = 2.35$$

Now look at the figure below to estimate $erfc\left(\frac{r}{\sqrt{4Dt}}\right)$



(the value is about 10^{-3})

Next insert the value of *Q* that you calculated earlier (which is $2.74 \infty 10^{-15}$) into the equation.

$$K = \frac{2.74 \times 10^{15} \text{ molecules/sec.}}{2(0.06)(3.14)(2)} \times 10^{-3}$$

$$= 3.63 \times 10^{12} \text{ molecules/cm}^3$$

Molecular Weight and Density (specific gravity) of Pheromones

Compound	Molecular Weight	Density
heptanol	130.23	0.8282
ethanol	46.07	0.7893
methyl heptanone	128.21	0.8304
hexane	86.18	0.65937
undecane	156.31	0.74017
formic acid	46.03	1.220
decyl acetate	312.54	0.8586
hexanal	98.15	0.8573
citronellal	154.25	0.8573

APPENDIX B
Supplies

Materials

Ants (*Pogomyrnex*, etc.)	Robins Scientific	
Reticulitermes	Carolina Bio	F6-59-2234
Forceps, Dumont No.5	VWR Scientific	63039-992
Hamilton Syringes	Fisher Scientific	14-824-3
Insect Pins	Carolina Bio	65-4300
Aluminum Foil	Carolina Bio	71-3210
Saran Wrap	Carolina Bio	71-3290
Screen Logo on T-shirts	Signature Apparel	
Petri Dish, 150x25	Fisher Scientific	08-757-145
Microflex Vials, 0.3	VWR Scientific	K-749000
Capillary Tubes	VWR Scientific	36984-003

Chemicals

Fluon AD1	Northern Products, Inc.	
(white fluid to prevent ant escape)		
1-octanol	Sigma Chemical	O 4500
Decyl Acetate	Sigma Chemical	D8759
4 Methyl 3 Heptanol	Aldrich Chemical	M4, 830-9
Methyl Heptanone	Sigma Chemical or Aldrich Chemical	
Ethanol	Sigma Chemical or Aldrich Chemical	
Hexane	Sigma Chemical or Aldrich Chemical	
Undecane	Sigma Chemical or Aldrich Chemical	
Formic Acid	Sigma Chemical or Aldrich Chemical	
Citronellal	Sigma Chemical or Aldrich Chemical	
Hexanal	Sigma Chemical or Aldrich Chemical	