INQUIRY & INVESTIGATION

I Walk the Line: A Popular Termite Activity Revisited

RECOMMENDED FOR *AP Biology*

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Abstract

Since 1968, biologists have known that termites line up and follow some ballpoint ink lines but not others. Suggestions for class lessons based on this observation have become widespread. However, many of these are incomplete, superficial, conflicting, and/or occasionally inaccurate, and most provide only simple demonstrations or cookbook-style confirmations. Here, we provide added background for this activity to update, clarify, and expand it. Some ways to use termite trail-following to teach fundamental life and physical science concepts through hands-on inquiry are presented, based on our experience with university students and teachers. These activities, adaptable to many instructional levels, range in scope from a single laboratory session to extended investigation.

Key Words: *Guided inquiry; Isoptera; chemical communication; 2-phenoxyethanol; pheromone; ink.*

In the years since the most recent review of the use of insects as teaching organisms (Matthews et al., 1997), many additional insect species and systems have migrated from the research laboratory to the classroom. Without a doubt, one of the most popular activities has been a simple exercise that involves termites and lines drawn with a ballpoint pen. Termite line-following has even captured attention on the Internet in the form of PDF files and YouTube videos. A simple Google query, "Why do termites follow ink," yields some 174,000 hits.

This attention is well deserved. Doing this activity engages students with real science, and the activity is easy, reliable, and inexpensive. However, much of the published and online information about it is redundant, inaccurate, or conflicting. Very few of the activity suggestions are inquiry based, and none provide a history of the activity or an entrée into pertinent entomological literature. Thus, it seems appropriate to update and extend this activity, including suggestions for new hands-on inquiry-based activities adaptable to various levels of instruction.

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○ Background

Any of the common subterranean termites in the genus *Reticulitermes* (5 species are recorded from the United States) is appropriate for this activity. These termites occur in all but the highest elevations and most arid areas of the United States. Quantities can be collected easily during the warmer months from decaying damp logs or other wood debris in forests and parks. One can simply shake infested wood over a plastic wastebasket or storage container to dislodge the insects. (Smooth plastic container sides are difficult for termites to walk upon, ensuring that they do not escape.) Care requires only daily misting with a water spray bottle and provision of some damp wood pieces for the termites to eat. Groups of collected termites will survive for some time under these conditions. Alternatively, living termites are available from Carolina Biological Supply Co. (Burlington, NC) and Ward's Natural Sciences (Rochester, NY).

Collected termites will be of two forms. The most numerous are the sterile workers, which have small, soft heads; the less abundant soldiers have distinctly larger, darker heads and strong mandibles (Figure 1). Termites are among the most social of insects. An entire colony contains thousands of workers, hundreds of soldiers, and one or more fertile kings and queens; all are necessary for the group to survive and thrive. For this reason, worker termites that might escape or disappear during classroom activities pose absolutely no threat to wood in classroom buildings. More information on the biology of *Reticulitermes* can be found in Thorne et al. (1999).

Every day or night, a great many termite workers must leave the nest and search for new sources of food and water. Then, upon finding potential provisions, they must somehow relay a message back to the others to help in the harvest. A food-searching termite forager deposits scent marks as it wanders, a fact first experimentally

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Figure 1. (Left) *Reticulitermes*, North America's common subterranean termite; the individual with the larger, darker head is a soldier; both soldiers and workers are blind. (Right) Hidden termites have eaten away the wallboard shown exposed here.

Figure 2. (Left) Small disposable deli containers are useful for holding termites between experiments. (Right) A small brush or chenille stick makes a good handling tool; any small plastic container with smooth sides such as a weighing boat will block termites from crawling out. However, without a lid, termites will soon desiccate.

demonstrated fewer than 40 years ago (Tschinkel & Close, 1973). Even if it does not find food, the termite can follow these scented droplets homeward. However, a successful termite returning home not only follows these droplets but also adds to its trail, laying down more droplets at greater concentration and at more frequent intervals. Other individuals that the worker encounters are stimulated to track the odor to the food source, reinforcing the track with their own scent marks. Over a relatively short time, these droplets become a more-or-less continuous chemical communication trail.

The chemistry of these communication chemicals, called pheromones (Wyatt, 2003), is now known for at least 60 termite species. Every termite species makes its own particular multicomponent cocktail (see reviews by Costa-Leonardo et al., 2009; Bordereau & Pasteels, 2011) and secretes it from glands located on the underside of their abdomens. The ingredients in these blends belong to three classes – alcohols, aldehydes, and hydrocarbons – but a major component found in several species is the alcohol (Z,Z,E)-3,6,8 dodecatriene-1-ol [= dodecatrienol]. This compound is not commercially available, but it has been synthesized.

The first observations that subterranean termites in the genus *Reticulitermes* follow lines drawn with ink pens occurred at the U.S. Forest Service Laboratory in Gulfport, Mississippi (Becker & Mannesmann, 1968). The earliest published teaching activity based on this behavior was only three paragraphs long, but it concisely captured the essence of an inquiry approach (Shanholtzer & Fanning, 1991).

○ Teaching Tips

Termite workers can live for several days in a lidded plastic dish provided with a bit of moist paper towel (Figure 2). Provide each group of students with two such containers for temporary storage, one as a source of experimental termites and the other as a repository for used individuals. Individual termites should be used only once in a given student experiment. However, the same set of termites can be reused by different groups in subsequent classes.

For best classroom management, dispense individual termites onto sheets of plain white copy paper. Wrangling termites is an acquired skill but is easily mastered. Place the bristles of a small, soft brush or the side of a short length of chenille stick beside the termite. With a rolling/sliding motion of the hand, carefully scoop the termite onto the bristles or fuzz. Gently tap to dislodge the termite; to place a termite in a precise spot, use a gentle reverse roll. When finished, move the paper to the edge of the table so that it slightly overhangs and use the brush or chenille to gently scoot the termites over the edge into the container.

To promote guided inquiry, minimize overly specific procedural advice and restrict guidance to that necessitated by limitations in readily available materials. We have found a modified five-stage learning-cycle approach – Engage, Explore, Explain/Elaborate, Extend/Expand, and Evaluate – effective with this activity (see Bybee et al., 2006, and references therein).

1. Engage

Show students living termites and photos or videos of termite damage. Are termites "good" or "bad"? Encourage discussion about the overly simplistic, context-dependent nature of such questions. Subterranean termites are among nature's premier recyclers. Most eat dead vegetation, breaking down materials that would otherwise accumulate. However, about 10% of the world's 2700 species are considered pests because their foods include buildings, crops, and plantation forests. Collectively, subterranean termites are responsible for considerable property damage, ranking them as one of the most destructive structural pests in the United States. Obviously, humans would love to find a way to reduce or prevent termite damage. One step toward that goal would be to determine how they congregate at a food source. Does each individual simply wander around until it chances onto something? Or can a successful forager communicate the location of its discovery and recruit other termites to join in plundering it?

2. Explore

Give each student or group a holding container, brush or chenille stick, and piece of blank paper. Dispense 3–5 worker termites onto each paper and allow students to observe the termites' behavior, using the brush to gently restrict the termites to the paper should any begin

Table 1. Black ink pens selected to show a range of termite responses.[a]

Pen Type	Brand & Model	Do Termites Follow the Ink Line?
Ballpoint	BIC Ultra Round Stic Grip	Yes
	BIC 4 Color Stylo-bille	Yes
	BIC Cristal	Yes
	BIC Ecolutions	Yes
	BIC Metal Point	No
	BIC Pro+ Stylo-bille	Yes
	BIC Atlantis Easy Glide	Equivocal results
	Papermate Grip	Yes
	Pentel RSVP	Yes
	Pilot G2	No
	Scripto Stick	Yes
	Staedtler Ball 432	Yes
Gel	BIC Velocity Gel	No
	Pentel Energel	No
	Uniball Gelstick	No
	Zebra Sarasa	No
Roller Ball	BIC Z4	No
	Uniball Vision Elite	No
Felt-tip	Pilot Precise V7	No
	Sanford Sharpie Permanent	No

[a]All types, brands, and models are available from Walmart, Office Max, and many other big box stores. Similar pens could be substituted as available.

to wander off. (This generally alleviates any student anxiety about handling the insects.) After 2–5 minutes, have students carefully return termites to their container and then report what they observed.

Pass around a box containing various colors and brands of pens, felt-tip markers, colored pencils, crayons, etc., so that each group gets an assortment of writing tools. Ask students to select a writing tool and use it to draw a complete, approximately fist-sized circle on their paper. (Termites will follow a tighter circle, but this size will give termites a chance to get their bearings before encountering the test line.)

Release 1–5 termites into the center of each circle and observe for another 2–5 minutes. Then return the termites to the holding containers. Each group should record and share their observations. Did termites cross the line and leave the circle? What differed between cases in which termites ignored the circle and those in which they followed it? Note that termites are liable to cross lines rather than follow them when they are first released and in the exploratory phase; the first response of any animal placed in new surroundings is to explore randomly before it "comes to its senses" and begins to respond to sensory inputs in a systematic way.

As a class, identify, list, and discuss the writing tools and colors associated with line following, repellency of lines, and random wandering. None of the marks left by the pencils, crayons, felt-tipped markers, rollerball pens, or gel pens will have been followed, but termites will have traced the marks drawn with many of the ballpoint pens. Table 1 shows results for selected inks that we have tested in classroom situations.

3. Explain

What appears to have happened? Develop a list of responses (for examples, see Table 2). Introduce some useful terminology (e.g., hypothesis, variables, etc.), pointing out that their observations have led to certain inferences about the cause(s) of the observed behavior. One can also use the ensuing discussion to introduce or

Table 2. Some student-generated observations, inferences, and suggestions.

Observation	Inference	Possible Tests
Termites follow some ballpoint pen lines but no other marks that we tested.	Something about ballpoint pen ink is attractive to termites.	Find out what is in ballpoint pen ink, and test the various chemicals with termites.
Termites follow some colors but not others.	Termites can see colors; the colors are chemically different.	Substitute paths cut from colored paper; use microscope to see whether termites have eyes.
Termites sometimes swing their heads from side to side while following trails.	Termites are using their antennae to stay where chemicals are rising from the trail; termites smell the chemicals; termites are looking around.	Clip antennae or cover with a blocking substance, then see what happens; use microscope to see whether termites have noses or eyes.
Even with "successful" inks, termites follow some better than others.	Termites are attracted by some component of the ink, not by the whole mixture; age of the pen matters.	Learn the ingredients in various pens, and compare them; make lines with old and new pens of same type.
Over time, termites don't follow a trail as well.	Finding no food reward, termites no longer "pay attention" to the trail; something about the ink is changing over time; the attractive component is something that evaporates.	Put a fresh termite on the same trail that the older one no longer follows; vary trail age by marking lines at different time intervals.

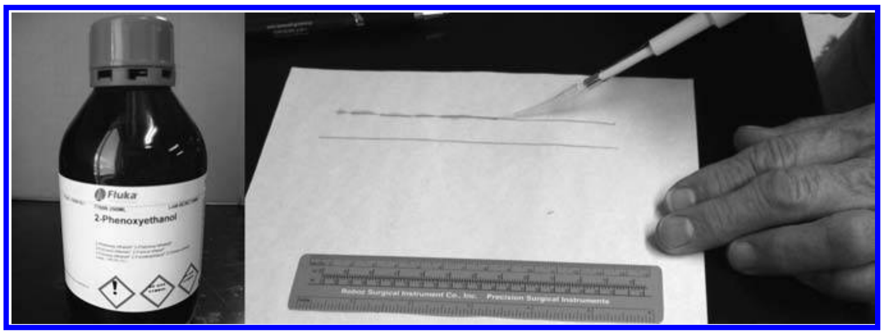

Figure 3. (Left) This 500-mL bottle of 2-phenoxyethanol contains enough active ingredient to lay 7,326,525 m of trail – about 18% of Earth's circumference! (Right) Properly diluted with 100% ethanol, PE can be pipetted onto a line as "invisible ink." Commercial availability from Sigma Aldrich opens a range of possibilities for inquiry.

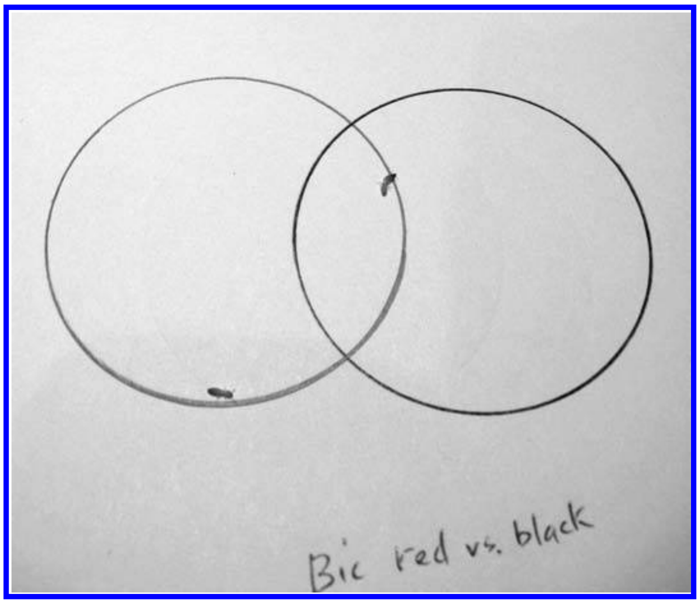

Figure 4. One student's simple design for determining the relative attractiveness of two ink colors of the same brand. Termites were placed in the overlap between the circles, and choices at the intersections were recorded.

reinforce the scientific method, if appropriate. This activity can be adapted and effectively used as the first formal laboratory activity of the year at any level (Surmacz, 2004; Johnson, 2009; Callis et al., 2010).

For student guesses or inferences to become working hypotheses, we find that it helps to formalize them into concise "If…then" statements. (For example, "If I place a group of termites in the center of a circle drawn by a blue ballpoint pen, then the termites encountering the circle will follow it.") Such a statement clearly designates both the independent (manipulated – ink circle of blue pen) and dependent (responding – termites following ink line) variables, and makes it immediately obvious when students are attempting to study more than one independent variable at the same time.

It is often suggested that termites follow inks that mimic their natural trail pheromones. However, the connection between termite trail pheromones and artificially produced ballpoint inks is more elusive than simple demonstrations of the phenomenon would suggest. It was 30 years after the original discovery before 2-phenoxyethanol (PE) was isolated from Papermate brand pen ink and shown to elicit trail following (Chen et al., 1998). The chemical formula for PE is $C_6H_5OCH_2CH_2OH$. Even though both the natural trail pheromone (dodecatrienol) and PE are primary alcohols, PE is not particularly structurally similar to dodecatrienol.

A colorless, oily liquid (Figure 3), PE is often used in formulations of dermatological products. Although some termite activities include conflicting reports that only certain brands of pens (usually said to be either Papermate or BIC) elicit trail-following, PE has actually been found to be present at some concentration in 85% (237/279) and 83% (293/354) of currently available black and blue ballpoint pen inks, respectively (Cantu et al., 2004). However, Cantu's study was designed strictly for forensic purposes and did not address termite responses. Subsequent research on the best PE concentration (0.23 µL/cm) for optimal trail-following (Fei et al., 2005) suggests that the attractiveness of various pens may be related to the PE levels that they contain.

4. Expand/Elaborate

Ask each student or group to think about ways in which they might determine whether their current hypotheses are supported. Encourage students to come up with different new hypotheses, varied experimental designs, and diverse data-recording systems. Then ask them to outline one experiment to test one hypothesis (see example, Figure 4). This should include a list of materials needed, a list of variables to be kept constant, and a simple data sheet on which to record results. This can be a homework assignment or an in-class activity – working in groups provides opportunity for productive interaction and may enhance the rigor of their experimental designs.

Allow time for each group to present their hypothesis and proposed experimental procedures. Solicit class feedback before allowing any experiments to begin. Depending on student level and curriculum constraints, encourage as much further experimentation as possible. Termite trail-following encompasses many potentially researchable questions. Table 3 provides some examples.

5. Evaluate

Instructors may wish to design a scoring rubric that emphasizes concepts related to explanations for observed behaviors and how well students use evidence to support claims. For further guidance on rubrics, see http://www.teach-nology.com/tutorials/teaching/rubrics/ or http://rubistar.4teachers.org/index.php.

We often ask our students to present their findings in a formal laboratory report, including the typical sections of a standard scientific research paper (Matthews & Matthews, 2008). Formal oral or PowerPoint presentations prepared by different groups are an alternative to a written report. In our experience, such techniques work well even with younger students, who respond very thoughtfully when they attempt to explain their data and consider potential practical applications.

Table 3. Some potential inquiry extensions of the termite activity.

Question	Experiment	Notes
Do soldier termites also follow ink trails?	Repeat experiments with a group of soldiers.	Soldiers follow odor trails well, often more persistently and reliably than workers.
How long before a trail evaporates?	Use lines traced at different times with the same pen.	Many factors affect evaporation rates. Runcie (1987) also has reported that *Reticulitermes* produce different trails persisting from 15 minutes to a year.
Do trails encode directional information?	Cut a section of paper with an actively followed trail, reverse it, reconnect it, add termites.	To date, little evidence for trail directionality has been discovered.
How tight an angle can a termite follow successfully?	Test termites on connected lines, varying the angle between them.	Termites often overshoot trails that require an acute-angle turn.
Do termites habituate to ink lines over time? Or does ink just "get old"?	When termite wanders, move it to a newly drawn line. Does it begin to follow again?	Accurate dating of ink is an important topic in crime cases involving things like ransom notes (see Cantu et al., 2004; Weyermann et al., 2007).
What concentration of PE makes the best trail?	Introduce PE as "invisible ink" (see Figure 1). Develop an operational definition of trail following (e.g., must follow for at least 10 cm).	Additional science skills (performing math conversions, micropipetting, making serial dilutions, etc.) will be reinforced in this extension.

○ Conclusion

Increasing students' understanding of how scientists work to ask appropriate biological questions and seek answers through research is a major goal of science teaching. Skills honed and nurtured through this authentic science activity enhance the development of content knowledge as well as scientific process skills, including observation, prediction, developing and testing hypotheses, data collection, and inference. This activity also can serve as a springboard for discussion of other fascinating and relevant topics related to insect biology and human cohabitation with insects.

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