SYMPOSIUM

Student Journal of Science & Math

Volume 2 Issue 1

biology
117
B
82.731

OBSERVATIONAL LEARNING IN EUSOCIAL INSECTS

A RESEARCH PROPOSAL

by Avity Norman

Background

Ants (order Hymenoptera, family Formicidae) are eusocial, meaning that they exist in colonies of reproductive (queen) and nonreproductive (worker) individuals with overlapping generations and cooperative care of offspring. Eusocial colonies have been called "superorganisms"—a level of complexity above the individual multicellular organism (Johnson & Linksvayer, 2010). Within a colony, complex communication and behavior coordination is accomplished primarily through chemical signals, which may be either airborne pheromones or cuticular hydrocarbons requiring direct ant-to-ant touch for transmission (Lahav et al., 1999; Johnson & Linksvayer, 2010). One of these cuticular hydrocarbon signals is a colony recognition marker, allowing individual ants to recognize other individuals as either nestmates or aliens—"self" or "non-self" (Lahav et al., 1999). Almost all ants respond either violently or avoidantly to ants from non-self colonies, regardless of conspecificity (Fowler, 1977).

Although ants of different colonies are hostile to each other, the nature of their communication may allow the possibility of involuntary information sharing. If chemical information can be disseminated between distinct colonies, this would be a form of observational learning, hitherto undiscovered in eusocial insects. To date, observational learning in invertebrates has only been studied in the cephalopods, which have the most human-like intelligence of all invertebrates (Fiorito & Scotto, 1992; Huang & Chiao, 2010). The cognition of eusocial insects is more alien to us, but these invertebrates exhibit staggeringly complex behavior indicative of great intelligence despite their individually tiny brains. The proposed study seeks to discover if ant colonies are capable of observational learning, and to improve our understanding and appreciation of the great and complex collective intelligence of these fascinating animals. It may also provide some insight into the evolutionary success of ants, as observational learning may improve fitness by sparing the learner the dangers of trial and error.

Leafcutter ants of the genus *Atta* feed only on a symbiotic fungus that they cultivate inside their nests

and feed with a variety of leaves. This genus is ecologically and economically important in the tropics of Central and South America, where its leaf-harvesting has a tremendous impact on vegetation (Moutinho et al., 2003; Costa et al., 2008). In general, *Atta* species are typical of Formicidae in terms of their chemical communication, and they have been shown to exhibit hostile behavior to ants from different colonies (Fowler, 1977). *Atta* species exhibit some of the most complex colonial organization systems of any eusocial animal with division of labor amongst four anatomically and behaviorally distinct castes of workers (Wilson, 1980).

Atta also lends itself well to ethological studies because its foraging behavior is easily observed and manipulated. Saverschek et al. (2010) used fungicide-treated leaves to induce an avoidance response to certain plants, and to demonstrate experiential learning and long-term olfactory memory in *Atta columbica*. The fungicide, cycloheximide, is imperceptible to the ants themselves but has a detrimental effect on the symbiotic fungus that is their sole food source. Saverschek et al. showed that *A. columbica* demonstrated colony-wide avoidance behavior within 24 hours of receiving fungicide-treated leaves, indicating a learned behavior transmitted throughout the colony by chemical communication. If these chemical signals can be transmitted between colonies, then an untreated colony exposed to a colony in which an avoidance response has been induced should begin to exhibit the same avoidance behavior.

Hypothesis

Hypothesis: Learned information can be transmitted between ant colonies.

- Sub-Hypothesis 1: Learned behavior can be transmitted between colonies by airborne pheromone exchange.
  - Prediction: A colony presented with only untreated leaves will exhibit induced avoidance behavior if allowed to exchange air with a colony that is being presented with treated leaves.
- Sub-Hypothesis 2: Chemical long-term memories can be transmitted between colonies by airborne pheromone exchange.
  - Prediction: A colony without avoidance behavior induced will exhibit avoidance behavior if allowed to exchange air with a colony in which avoidance behavior has been induced.
- Sub-Hypothesis 3: Chemical long-term memories can be transmitted between colonies by direct contact between individuals.
  - Prediction: A colony without induced avoidance behavior will exhibit avoidance behavior if allowed to make direct contact with individuals from a colony in which avoidance behavior has been induced.

Project Objectives

Objective 1: Locate four colonies of *Atta columbica* on Barro Colorado Island. Assess baseline foraging activity and colony size.

Objective 2: Establish two formicaria with workers collected from Colony #1. These will be called 1A

and 1B.
Objective 3: Install Formicarium 1A in the territory of Colony #2 and Formicarium 1B in the territory of Colony #3, so that they can exchange air but cannot make direct contact between individuals. Allow acclimation time.
Objective 4: Use fungicide (cycloheximide) to induce *Stigmaphyllon lindenianum* avoidance in Formicarium 1A.
Objective 5: Monitor acceptance/avoidance of *S. lindenianum* in Colony #2.
Objective 6: Transplant a number of individuals from Formicarium 1A into Colony #4. Monitor Colony #4 for *S. lindenianum* acceptance/avoidance.
Objective 7: Move Formicarium 1A into the territory of Colony # 3, replacing Formicarium 1B. Monitor for *S. lindenianum* acceptance/avoidance in Colony #3.

Methods

Colony Selection
Four mature colonies of the leafcutter ant *Atta* colombica will be located on Barro Colorado Island, Panama, a species-rich tropical moist forest (Saverschek et al., 2010). These colonies will be numbered 1-4. A capture-mark-recapture technique will be used to estimate the size of each colony from the number of foragers. Colonies selected should be of medium size with 10,000 to 50,000 foragers each. Foraging activity in each colony will be assessed using a sugared-oat pick-up test and refuse dumping rate as described by Saverschek et al. (2010).
Two formicaria, labeled 1A and 1B, will be collected from Colony #1 and housed in the laboratory for 3 weeks. Each formicarium should contain 1,000 to 2,000 foragers with proportionate numbers of other workers, fungus garden, and nursery housed in a portable assemblage of plastic bins and tubing.

Experimental Setup
Formicarium 1A will be installed within Colony #2, separated in every direction by a double layer of fine mesh to allow exchange of air while preventing direct contact between individuals. Formicarium 1B will be likewise installed within Colony #3. Both colony setups will be maintained for 3 weeks before experimental procedures are begun to allow the formicaria and host colonies to acclimate to each other's presence. All formicaria and colonies will be given untreated discs of *Stigmaphyllon lindenianum* and *Spondias mombin*—two common, well-accepted feed plants—during this time.

Experimental Procedure
After 3 weeks, Formicarium 1A will be presented with discs of *Stigmaphyllon lindenianum* infiltrated with the fungicide cycloheximide (CHX) by the method described by Herz et al. (2008). Saverschek et al. (2010) demonstrated that this fungicide, though imperceptible to the ants themselves, induces a delayed rejection response in *A. columbica* within 24 hours due to its detrimental effects on the symbiotic fungus. This avoidance behavior will be monitored in both Formicarium 1A and its host, Colony #2. After avoidance behavior has been induced in Formicarium 1A, the ants will no longer be presented with

CHX-treated leaves. Saverschek et al. (2010) showed that olfactory long-term memory maintains the induced avoidance behavior for up to 18 weeks. To test whether this long-term memory can be picked up by another colony via direct contact between individuals, 200 to 300 foragers from Formicarium 1A will be transplanted directly into Colony #4. Colony #4 will then be monitored for acceptance or avoidance of *S. lindenianum*. To test if the long-term memory can be shared between colonies by airborne pheromone exchange, Formicarium 1A will be moved into Colony #3, replacing Formicarium 1B, and separated as before by a double layer of fine mesh. Because both formicaria are taken from the same field colony and Colony #3 has been given time to acclimate to the presence of a non-self formicarium, the only difference should be the presence of an avoidance memory in the formicarium. Colony #3 will be monitored for acceptance or avoidance of *S. lindenianum*.

Data Analyses and Expected Outcomes

To monitor for avoidance behavior in each formicarium or field colony, the numbers of *Stigmaphyllon lindenianum* and *Spondias mombin* leaf discs picked up by *Atta columbica* foragers on each of four foraging trails will be counted for six 30-minute periods per day for two days before and four days after the introduction of CHX-treated *S. lindenianum* leaves or of *S. lindenianum*-avoidance-induced ants. Intake rate will be defined as the mean number of leaf discs picked up on one foraging trail in 30 minutes. Repeated-measures ANOVA will be used to compare the intake rates for the six days of observation in each colony and in Formicarium 1A.

Formicarium 1A is expected to show a significantly decreased *S. lindenianum* intake rate and no significant difference in *S. mombin* intake rate after the introduction of cycloheximide-treated *S. lindenianum* leaf discs.

If the learned avoidance behavior can be transmitted between colonies by airborne pheromones, Colony #2 is expected to show a significantly decreased *S. lindenianum* intake rate and no significant difference in *S. mombin* intake rate after the introduction of cycloheximide-treated *S. lindenianum* leaf discs to Formicarium 1A.

If long-term avoidance memory can be transmitted between colonies by airborne pheromones, Colony #3 is expected to show a significantly decreased *S. lindenianum* intake rate and no significant difference in *S. mombin* intake rate after the introduction of Formicarium 1A.

If learned avoidance behavior can be transmitted between colonies by direct ant-to-ant contact, Colony #4 is expected to show a significantly decreased *S. lindenianum* intake rate and no significant difference in *S. mombin* intake rate after the introduction of individuals from Formicarium 1A.

Timeline and Budget

Timeline

Tasks:	Week:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Order, receive, and assemble materials																		
Objective 1																		
Objective 2																		
Objective 3																		
Objective 4																		
Objective 5																		
Objective 6																		
Objective 7																		
Write-Up																		

Budget

- Airfare to Panama City for lead researcher and 3 student assistants: $4,000
- 3 months' food and lodging at Barro Colorado Island for lead researcher and 3 student assistants: $7450
- Barro Colorado administrative fees: $250
- Formicarium materials: $300
- Cycloheximide: $100
- Lead researcher stipend: $4000
- 3 student assistant stipends: $3000

TOTAL: $19,100