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AMERICAN COCKROACHES EXHIBIT INTER-INDIVIDUAL VARIATION IN RECEPTIVITY TO CLASSICAL CONDITIONING

Lyndsay S. Ricks, Donald H. Feener, Jr.
School of Biological Sciences

Abstract

The American cockroach (*Periplaneta americana*) has occasionally been used as a behavioral model in entomological research, and has been demonstrated to be capable of associative learning. Additionally, consistent inter-individual behavioral differences among different cockroach species have been observed and termed "personality differences." I attempted and was unable to demonstrate that a correlation existed between facets of cockroach personalities and individual cockroaches' ability to learn to associate a location with a positive stimulus. According to my results, there nonetheless exists substantial inter-individual variation in learning ability, and prior behavioral research on *Diploptera punctata* demonstrating differences in "boldness" appears to be extensible to *P. americana*. This has implications for future research on cockroach behavior.

Introduction

Periplaneta americana is a common pest species of cockroach found worldwide, often used as a behavioral model in research due to its ease of rearing and acquisition. Previous research has demonstrated that *Periplaneta americana* and other cockroaches are able to form counter-instinctual behavioral associations and alter behavior based on learning. Other research has indicated that individuals of *P. americana* and of related species *Diploptera punctata* and *Blattella germanica* have what may be referred to as "personalities," that is, consistently observable inter-individual variations in behavior. My goal with this study is to, building upon previous research in these fields, examine whether there exists a connection between individuals' personalities and the way in which they learn (in this case, specifically the way in which they form spatial associations).

Prior research has established that when exposed to combinations of stimuli such as flashing lights and odors (Lent and Kwon 2003) and odors and tastes (Sakura and Mizunami 2001; Watanabe and Mizunami 2007), *P. americana*'s behavior can be conditioned to acclimate to these combinations. As one example, Sakura and Mizunami (2001) exposed specimens to an unattractive peppermint odor in combination with an attractive sucrose solution and an attractive vanilla odor in combination with an unattractive NaCl solution; they found that cockroaches quickly began to associate peppermint with a reward and vanilla with a non-reward, despite their instinctual tendency to prefer the obverse. The same study also indicated some level of variation in the intensity of the effect produced by this treatment, i.e., apparent variation in the cockroaches' learning ability.

In addition to conditioning responses, several species of cockroach have been demonstrated to possess what might be termed "personalities." *Periplaneta americana* exhibit differences in

thigmotactic preference (Laurent Salazar *et al.* 2018), meaning the degree to which individuals gravitate towards walls and enclosed spaces, as well as differences in individual sheltering probability (Planas-Sitjà *et al.* 2015). The distantly related cockroach *Diploptera punctata* also exhibits cross-individually differing degrees of "boldness," i.e., consistent results between multiple emergence tests within one individual (Stanley *et al.* 2017), although similar studies have not apparently been attempted on *Periplaneta americana* or any other blattids.

I have made the following hypotheses in light of this:

i. The consistent differences in boldness demonstrated in *Diploptera punctata* can also be observed in *Periplaneta americana.*
ii. *Periplaneta americana* sheltering probability and thigmotaxis will both be negatively correlated with boldness (i.e., an increase in boldness will mean a decrease in sheltering probability and thigmotaxis).
iii. *Periplaneta americana* should be capable of undergoing classical (Pavlovian) conditioning in order to associate a particular location with a food reward.
iv. Individuals exhibiting higher levels of boldness will be conditioned to associate location with reward more quickly than individuals with lower levels of boldness.

In order to test these hypotheses, I adapted the methods of Stanley *et al.* (2017), Planas-Sitjà *et al.* (2015), and Laurent Salazar *et al.* (2018) to test individual subjects' boldness, sheltering probability, and thigmotaxis respectively. In order to examine learning, the time taken for subjects to reach a food incentive (placed in the same location each time) on an open field over five consecutive days was recorded and analyzed.

Materials and methods

Subjects

The subjects consisted of sixty adult male specimens of *Periplaneta americana* were obtained from Benzon Research (Carlisle, PA) in August 2019. Specimens were identified by small plastic strips numbered from 1 to 60, which were attached to the pronotum with medical latex adhesive (cockroaches were sedated with CO_2 immediately prior to application). Subjects were randomly allocated to two separate glass terrariums (dimensions 51.435 cm × 26.67 cm × 31.91 cm), which each contained one cardboard cup hide and an approximately 1.5 cm thick layer of coconut fiber substrate. The top eight centimeters of the wall was coated with petroleum jelly to prevent climbing. Subjects were given koi pellets (Blue Ridge Koi) *ad libitum* and the terrariums were misted on Mondays, Wednesdays, and Fridays to provide subjects with water.

Twenty-six subjects were excluded from final analysis – in most cases, this was due to death from natural causes, although several subjects were excluded because they sustained unexplained physical damage or because unfixable errors occurred during the administration of the experimental treatments. In total, thirty-four of the original sixty subjects were included in analyses.

Thigmotaxis tests

In order to obtain thigmotaxis data for cockroaches, a heavily modified version of Laurent Salazar *et al.*'s (2018) method was employed. The same basic layout of the container used for testing thigmotaxis was used: a lidless cylindrical acrylic container (Azar Displays, Kingston, PA) with a 25.4 cm diameter and measuring 15.24 cm tall, was employed as the testing arena. The walls of the container were fully coated with petroleum jelly to prevent escape and the floor was lined with black construction paper, which was changed between subjects. A thick layer of

red cellophane was stretched over the top of the container in order to decrease light penetration; this was lifted up as necessary to begin treatments. Cockroaches were continuously kept in low light conditions (a dim red light was on in the room in which they were stored) prior to treatment.

Individual cockroaches were obtained from their terrariums and placed in the center of the arena, then the light was fully turned off so that the subject wasin complete darkness. The red light was briefly turned on every three minutes after the start of the trial to observe the behavior of the subject. Its position was recorded via overhead webcam if it was resting (not moving any body part except the antennae), and otherwise, the light was immediately turned off for another three-minute cycle. Cockroaches were removed and returned to their terrariums after their initial resting position was determined. Images were then adjusted to improve contrast and mapped to a circle using Inkscape so that the angle of the cockroaches (relative to the center and relative to the cerci) could be appropriately measured. Two non-consecutive trials were performed per cockroach, all between 8:00 AM and 6:30 PM.

Sheltering tests

A modified version of Planas-Sitjà *et al.*'s (2015) method for examining individual and group sheltering time was used to test the sheltering preferences of the individual cockroaches. The same circular container from the thigmotaxis tests was reused, so cockroaches were somewhat acquainted with their surroundings. However, in contrast to the thigmotaxis trials, a tube-like hide (r = 3.175 cm, h = 2.54 cm) made of cardboard and enclosed by black construction paper, with a thick layer of red cellophane stretched across the top (allowing subjects to be visible in recordings while providing an adequate low-light area) and two 30° long, 1.5 cm tall openings on opposing sides, was placed in the center of the arena. The arena was lit by a bright white light rather than red light in order to provide an incentive to shelter, and white paper towels were placed on the ground and changed between subjects. Cockroaches were taken from their standard enclosures using a small deli cup and immediately introduced to the arena by placing them on top of the hide. Cockroaches were observed for 30 minutes after introduction and their total sheltering time during the 30-minute period was recorded. Two non-consecutive trials per cockroach were performed, all between 8:00 AM and 6:30 PM local time.

Boldness tests

In order to test boldness, a very similar method to that used by Stanley *et al.* (2017) was employed. Cockroaches were kept on a 12:12 L:D photocycle prior to performing the test. Initially, an emergence test was performed: subjects were placed in and acclimated within a 6 cm-diameter and 5-cm long section of a cardboard mailing tube, fully encircled by black construction paper in order to minimize light input. They were kept within the tube for four minutes and then released into the left side of a well-lit plastic container with whiteboard paper on the bottom, with a fully proportional 5x4 grid drawn on it (cells labeled using red marker to limit marking visibility). The container's walls were fully coated in petroleum jelly to prevent cockroaches escaping. Clear laminating sheets were placed over the bottom and changed between subjects to avoid cross-contamination while providing grid visibility. Cockroaches were recorded for 10 minutes, or until all cells had been visited, after the 4-minute acclimation period. From analyzing the videos, the time it took for subjects to fully exit the tube (i.e., all six feet in a cell other than B1 or C1) was recorded, as well as a list of cells visited by each one. This experiment was performed and results were recorded twice. No cockroach was tested twice in the same day and all tests were performed some time between 8:00 AM and 6:30 PM local time.

Conditioning

For the conditioning treatments, cockroaches were placed in the same container as the boldness tests, except that they were placed in the container via deli cup rather than mailing tube and the grid was erased, so that the only marking present on the ground was a small red dot on the opposite side of the entry point to provide a guide for the location of the enticement. Laminating sheets, allocated to individual subjects and reused between days but not between subjects, were used to cover the whiteboard paper. They were thoroughly rinsed with water between treatments in order to prevent the cockroaches from using their own scent marks to find their way to the reward (see Jeanson and Deneubourg 2006). Treatments were performed under red light.

Cockroaches were separated into three arbitrary groups between ten and fifteen individuals each, with each being designated to undergo conditioning on a particular week in November 2019 (these groups were designated for convenience, not significance). Cockroaches were deprived of food during active treatments and given a small quantity of fish pellets on the Sunday prior to treatment. On Monday of each subject's designated week, the subject was placed in the container described above, with no enticement. An overhead webcam was used to record the behavior of the cockroach for ten minutes or until it touched the red marking. On Tuesday through Friday, an enticement in the form of a drop of organic honey was placed on the target and cockroaches were again recorded for ten minutes, until they began eating the honey, or until they visibly passed over the honey three times without eating it (considered a refusal of the enticement). All tests were performed between 7:00 PM and 11:00 PM, as prior research indicates that cockroaches better retain memory of conditioning when the treatments are performed in the early subjective night (Decker *et al.* 2007; Page 2009).

Figure 1: A simple representation of the arena used for conditioning exercises (cockroaches were added to the container on the side of the deli cup and the enticement was placed on the top of the red dot).

Results

Thigmotaxis

The distance from the center that cockroaches elected to rest during thigmotaxis tests was not consistent across trials; no significant correlation existed between the results of the first and second trials ($p > 0.05$, $r^2 = 0.00539$). Since this indicates that the measurements taken were not reliable, thigmotaxis measurements were not included in any further analysis.

Sheltering

As with thigmotactic preference, the total amount of time that cockroaches chose to rest was not consistent across trials ($p > 0.05$, $r^2 =$ 0.05187). Again, these results were not strong enough to warrant inclusion in analysis.

Boldness

Results for emergence time in the boldness tests were somewhat correlated across trials (p = 0.01225, r^2 = 0.2258), although individual cockroaches sometimes exhibited behavior in the second trial that would not be anticipated from the first.

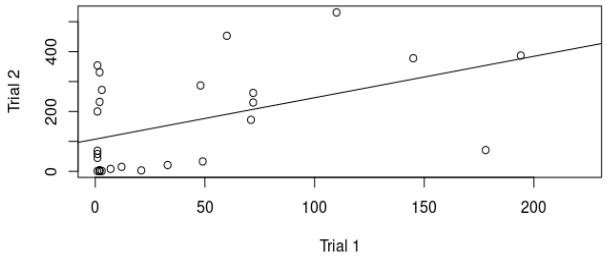

Figure 2: A plot of emergence time, in seconds, across trials. Each point represents a single subject.

Conditioning

The results yielded from conditioning treatments were very different from a standard expected learning curve, which in this instance would likely exhibit a linear or exponential decay in time taken to reach the enticement (the expected differences between cockroaches would be solely in the rate of this decay). However, subjects had wildly differing learning curves, featuring unexpected jumps between days and often ending up taking *longer* to reach the enticement at the end of treatment than at the start.

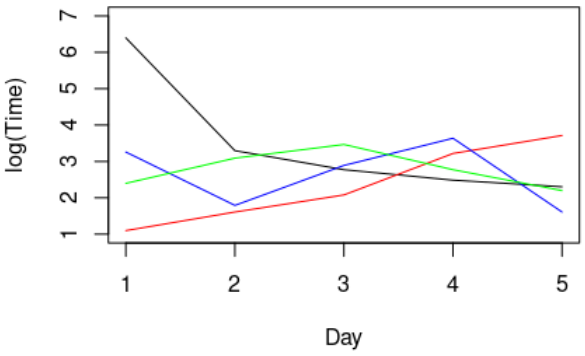

Figure 3: An illustrative example of four different cockroaches' (each indicated by a different color) learning curves.

In the graph shown to the left, the time it took for four different cockroaches to reach the enticement (log scale) per day is shown. The subject shown in black (subject 37) was the only subject to demonstrate the expected exponential learning curve, while three other cockroaches with heavily differing curves are shown to illustrate the amount of diversity present in responses.

Observations during treatments

While timing appears to be an unreliable means of measuring the effectiveness of conditioning for these cockroaches, I observed what seemed to be more directed and straightforward movement on the part of the subjects on days 4 and 5 (as opposed to days 1 and 2, which generally seemed to be more of a "directed random" walk). While I did not measure the actual movements of cockroaches due to time constraints and a lack of resources, an approach based on movement tracking might yield less diversity.

Boldness and learning

Two similar statistical techniques were employed to test the relationship between the outcome of learning treatments and the boldness of each cockroach: a multivariate analysis of variance (MANOVA) was applied with each day's recorded time modeled as dependent on emergence test results, and an analysis of covariance (ANCOVA) was also applied with each day's recorded

time modeled as covariant and dependent on the day of testing as well as subject boldness results. Neither technique yielded statistically significant results ($p > 0.05$ in both cases).

Discussion

The severe lack of consistency across trials for sheltering preferences and thigmotaxis appears to indicate a problem with the method used to determine these. In the case of thigmotaxis, the original study sedated cockroaches with CO_2 prior to introducing them to the test arena, which I was unable to replicate for logistical reasons, and is the likely cause of the failure to produce results. Cockroaches were not tested individually in Planas-Sitjà *et al.*'s (2015) original study on sheltering preferences, and subjects were tested within a much larger area, so one of these is the likely issue.

However, there was consistency in boldness, demonstrating that Stanley *et al.*'s (2017) research on *Diploptera punctata* is to some degree extensible to *Periplaneta americana* – that is, *P. americana* demonstrate "bold" personality traits in much the same way as *D. punctata*. While this does not appear to be connected to the way in which *P. americana* learn, it is regardless interesting, and it may imply that there is not much innate behavioral divergence across cockroach families, as the Blaberidae of *D. punctata* and Blattidae of *P. americana* are generally considered to be relatively phylogenetically distant within the Blattodea (Inward *et al.* 2007; Bourguignon *et al.* 2018). The lack of connection between boldness and learning ability according to my results is likely not a valid reason to discount the possibility of a connection existing, due to several issues with testing the learning ability of the cockroaches.

The diversity in observed learning trends between individuals may be indicative of one of several possibilities. First, this could simply represent a failure in experiental application or design: if this method simply does not work as a means of conditioning the cockroaches, then a random distribution would be expected (in particular, an additional stimulus may be necessary to induce learning in the cockroaches, such as a visual cue). Additionally, the cockroaches were likely fairly old at the time that conditioning was applied, which could potentially decrease its effectiveness, as age has been shown to impair learning and cognitive function in cockroaches (Brown and Strausfeld 2009). The cockroaches were obtained as adults, so their exact age at the time of testing is unclear, but the high incidence of mortality among the subjects despite the perfectly adequate living conditions seems to indicate that the general age was fairly old. Finally, it is certainly plausible that there is innate inter-individual variation in learning ability among cockroaches, and further research is certainly warranted to examine this.

Conclusion

There is still much research that needs to be done on the topics I have covered. I failed to find evidence of certain facets of cockroach personalities (thigmotactic and sheltering preferences) that has been found by previous researchers, but flaws in my make it impossible to discount that those exist, and studies correcting those flaws and searching more deeply are certainly warranted. I could not conclusively demonstrate that subjects were conditioned to expect a food reward if they went to a particular location in an open field, but the reason for this lack of a strong trend is yet to be made clear.

Of the four hypotheses I outlined initially, the first can be tentatively accepted: *Periplaneta americana* do indeed appear to exhibit a similar type of inherent "boldness" to *Diploptera*

punctata. As for the other three hypotheses, the null hypothesis cannot be rejected, as there is little evidence to support them at this juncture, but more research is needed.

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