Repeatability of *Peromyscus* Exploratory Behavior in the Hole-Board Apparatus

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1. Abstract

The past decade has drawn increasing attention to intra-individual variation in behavior in the context of natural selection. Temperament appears to be shaped by environmental heterogeneity, affecting dispersal, space use, diet, predator vulnerability and parasite load. Although the hole-board apparatus has long been used to test exploratory behavior in lab mice, it has yet to test wild mice, making it repeatability unknown. At the the University of Notre Dame Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan, 63 trials were run on 21 individuals of the genus *Peromyscus* between the 29th of June and 15th of July at to determine the repeatability of exploratory behavior on the hole-board apparatus. Behavioral assays measured three levels of exploratory behavior and looked at sex and species for factors.

2. Introduction

From little observation, individuals within a species can exhibit large variation in behavior even when categorized into age, sex, or size (Wilson et al. 1994). This phenomenon, often termed temperament, inter-individual variation in behavior that is consistent across spatio-temporal conditions, has drawn the attention of evolutionary biologists and behavioral ecologists throughout the last decade (Boyner et al. 2010; Réale et al. 2007). Recent studies indicate that such variation in behavior of wild populations is preserved because of fluctuating selection pressures introduced by environmental heterogeneity (Carere et al. 2010; Boyer et al. 2010). Individual temperaments are sensitive to natural selection pressures, for they have been shown to affect dispersal in great tits and passerine birds or space use in North American red squirrels (Wilson et al. 1994; Boyner et al. 2010). Wilson and his colleagues in 1993 also revealed that the shy-bold continuum affected diet, predator vulnerability and parasite fauna in juvenile pumpkinseed fish. Temperament is thus a balance between acquiring the benefits of resources and mates and protecting the self from danger in the form of predators and parasites (Wilson et al. 1994; Boyner et al. 2010). Temperament is thus usefully separated into 5 axis of personality, each defined according to a different ecological situation: shyness-boldness, exploration-avoidance, activity, aggressiveness and sociability. (Réale et al. 2007; Sih et al. 2004).

Although many studies have utilized the hole-board apparatus to quantify exploratory behavior in lab mice and rats for mainly pharmacological purposes, using the number of head-dips as a reflection for decreased anxiety and calmer emotional states, few studies have attempted to utilize the hole-board to test exploratory behavior in natural populations (File and Wardill 1975; Takeda et al. 1998; Biklei-Gorzó and Gyertyán 1996). To further study exploratory behavior would be to better understand the mechanisms of natural selection and the implications it has for ecology (Réale et al. 2007). Thus to be able to reliably quantify exploratory behavior in individuals is essential.

The genus *Peromyscus* is an ideal study system for exploratory behavior. The two, *P. maniculatus* and *P. leucopus* are one of the most abundant mammals in all of North America, spanning from the Atlantic coast to the Pacific coast, north to Alaska and south to central Mexico (Joyner *et al.* 1998; Ramsdell *et al.* 2008). *Peromyscus* varies widely in morphology, physiology, coat-color, behavior, growth pattern and diet allowing investigators to study natural genetic variation and adaptability (Ramsdell *et al.* 2008). They also serve as an important intermediate species between humans and two of the most widely-studied rodent models *Rattus norvegicus,* the Norway rat, and *Mus musculus*, the house mouse. Because *Peromyscus* readily adapts to laboratory colonies, parallel studies between wild-type deer mice and their laboratory counterparts are possible (Joyner *et al.* 1998). These and other studies on *P. maniculatus* have provided a greater understanding of ecosystems, evolution at the morphological level, biological rhythms and the epidemiology of many human diseases (Joyner *et al.* 1998; Ramsdell *et al.* 2008). *P. maniculatus* is an ideal study organism for many disciplines, earning itself the title "The *Drosophila* of North American Mammalogy" (Musser and Carleton 1993).

63 trials were run on 21 individual *Peromyscus* between the 29th of June and 15th of July at the University of Notre Dame Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan to determine the repeatability of *Peromyscus* exploratory behavior on the hole-board apparatus. Behavioral assays measured three levels of exploratory behavior and looked at sex and species for factors.

3. Material and Methods

3.1 Animals and husbandry

Seven field sites at the University of Notre Dame Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan were randomly selected. Five lines of five small mammal Sherman traps (17 x 5.4 x 6.5 cm) spaced 15 meters apart were set out one hour before sunset and were baited with a mixture of rolled oats and sunflower seeds (Ostfeld et al. 1996; Mather et al. 1989). All traps were checked in the early morning, with empty traps closed to be reset for the evening or kept open for the remainder of the day for on-going experiments. Upon capture, the sex and reproductive status of each mouse was determined. Previously untagged mice were tagged with a 4 digit ear clip for future identification. Furthermore, measurements of weight and body length were taken to the nearest gram and millimeter respectively. Animals were kept in plastic cages (19.05 x 21 x 12.70 cm) in the laboratory fume hoods for ventilation purposes and provided food and water *ad libitum.* Animals were treated in accordance to regulations implemented by the American Society of Mammalogists (Sikes *et al.* 2011) and the Animal Behavior Society and all protocols were approved by the University of Notre Dame

Institutional Animal Care and Use Committee under UND IACUC Protocol #xxxxxxx. Mice were released at the site of capture upon completion of behavioral assays.

3.2 Hole-board behavioral apparatus

Placed in a lidded 15 gallon aquarium (61cm x 31cm x 31cm) to prevent escape, the hole-board apparatus (29 x 29 cm) was made from Plexiglas, drilled with 16 evenly spaced bottom holes 2.0 cm in diameter, painted with a non-toxic, permanent white paint and raised on 9 cm tall wooden stilts. An acclimation chamber made of PVC piping 10.5 cm in diameter and 12.0 cm tall was also placed in the aquarium with a removable Plexiglas lid.

3.3 Procedure

Tests were conducted between 22:00 and 4:00 h using a hole-board test for three consecutive nights starting on the night of capture to fulfill a repeated measures experiment (Archer 1973; Walsh & Cummins, 1976). Prior to testing, subjects were gently moved into the lidded acclimation chamber. After an acclimation period of 10 minutes in the dark, subjects were given access to the hole-board by manual removal of the lid (Careau *et al.* 2011). Each two minute trial began at the time of landing and was video-recorded under infrared lighting with a Sony DCR-DVD610 DVD Handycam Camcorder in the Nightshot Plus mode located 2.5 meters away from the apparatus. A cut-off of ten minutes was used if the subject did not enter the hole-board (Palanza 2001). To control for lingering olfactory cues, the aquarium and hole-board apparatus was disinfected with 10% bleach between successive trials (Careau *et al.* 2011, Palanza 2001).

3.4 Behavioral analysis

The two-minute trials began upon the time of landing and three separate exploratory behaviors were scored off the videotape by the experimenters: latency to emerge i.e., the time from the removal of the lid to having completely left (four paws on the ground) the acclimation chamber; time to landing, defined as entry onto the hole-board with all four paws (10 min. maximum time); number of holes visited per second in sight.

3.5 Statistical analysis

For the reason that latency to emerge was a prerequisite to time of landing, time to landing was measured from latency to emerge to keep the two variable independent of each other. To control for out-of-sight exploratory behavior, the third measure was calculated as a ratio of holes visited per second of in sight exploratory behavior.

Variance in individual behavior will be controlled by performing a repeated measures ANOVA. A RM ANOVA using the data from 45 hole-board tests run on 15 individuals of the species *P. maniculatus* will determine whether there is a statistically significant difference in exploratory behavior of mice between trials. Factors that were also considered in the statistical tests included the sex of individuals. A second RM ANOVA will be run using the data from six *P. leucopus* and six randomly selected *P. maniculatus* to determine the effect of species on behavior as preliminary data for future experiments. Regression analyses for each measure of exploratory behavior was also run to determine the effect of time of trial on exploratory activity of individuals.

4. Results

4. 1 Intra-individual

A data set of 45 trials run on 15 *P. maniculatus* individuals was run through a RM ANOVA. Replicate behavioral trials exhibited no statistically significant differences in latency to emerge, time to landing, or number of holes visited per second across individuals and across sex. There however does appear to be an interaction between sex and latency to emerge (Fig. 1).

While the mean emergence times for males do not show a significant change across trials, females show a noticeable decrease in latency to emerge.

4.2 Inter-species and time effect

A data set of 36 trials run on six *P. maniculatus* and six *P. leucopus* individuals was run through a RM ANOVA. Replicate behavioral trials exhibited no statistically significant differences across all three levels of exploratory behavior and across species. The regression analysis used to determine the relationship between exploratory behavior over the time of night the trial was run revealed no trend.

5. Discussion

The lack of statistical significance between individuals in all three measures of exploratory behavior alludes to the precision of the assay, suggesting it as a powerful tool for measuring exploratory temperament. This has great implications for natural selection, ecology, and genetic studies. If the phenotype of exploratory behavior can be accurately determined by this hole-board apparatus, future experiments can isolate the gene for exploratory behavior and further understand what drives the mechanisms behind behavior.

Although there were no significant differences in any of the ANOVA analyses that were run, there appeared to be an interaction between sex and latency of emergence where females seemed are trending on exhibiting more exploratory behavior across trials with a pvalue=0.077862and males exhibiting no difference. Although the figure appears to significantly differentiate trials with large dips in values between the three trials and a small error bar on the last trial, the insignificance of the pvalue could be explained by the small sample size. Future experiments should aim to increase the sample size of both *P. maniculatus* to hash out possible interactions and of *P. leucopus* to observe inter-species behavioral differences.

6. Figures

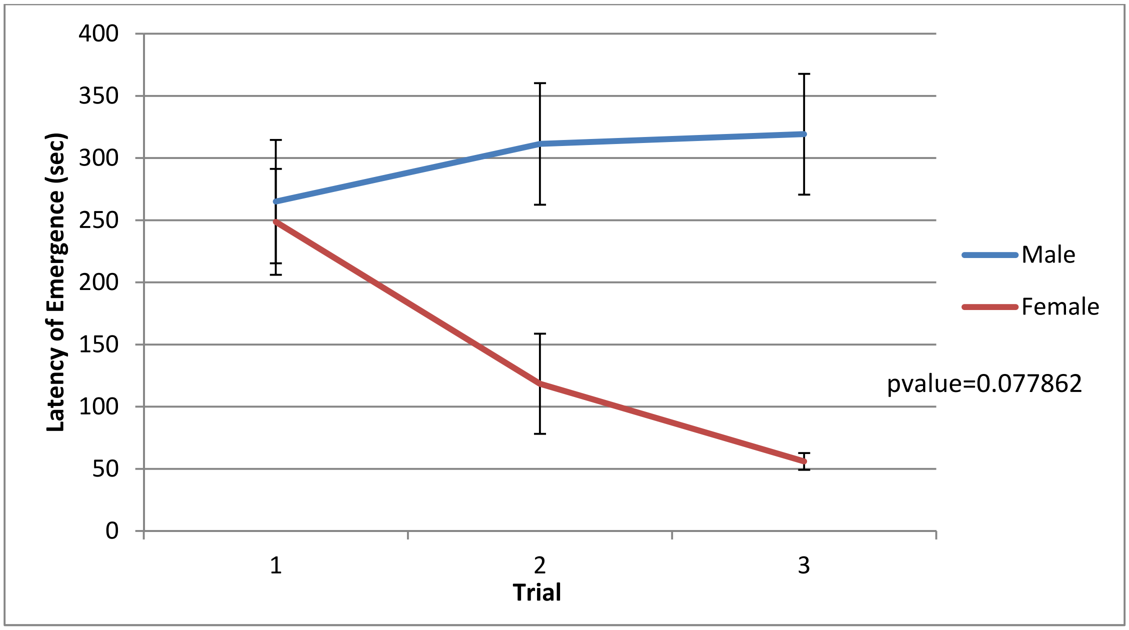

*Fig. 1*A RM ANOVA depicting the interaction between latency of emergence and trial number with sex as a factor. Where females appear to become more exploratory with a decrease in latency of emergence, males did not have a significant change. The statistically insignificant pvalue may be attributed to the small sample size.

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