An Experimental Approach to Studying Associative Learning in Harvestmen (Arachnida, Opiliones)

Introduction

The order Opiliones, generally known as harvestmen, is the third largest order of Arachnida, following Acari (mites and ticks) and Araneae (spiders). Despite this diversity, they are a very understudied order within the animal kingdom, this including studies of their learning behavior. Associative learning, the consequential learning from forming a relationship between two stimuli (Mackintosh 1983), has been investigated in harvestmen once by Dr.Hogan and his colleagues in 2013. Through this study, they found that harvestmen associated a chemical stimulus with a shelter. Although these findings are significant, further study, repetition, and methodological editing is required to validate these findings.

Harvestmen occupy a variety of niches where associative learning may be advantageous. As opportunistic and omnivorous foragers of small arthropods, worms, fungi, and other decaying organic materials (Giribet 2015), they can be expected to have some flexibility in their behaviors (Acosta and Machado 2007). They live in complex environments, such as the tropical habitat of the species of study, *Discocyrtus invalidus.* Occasional alterations in the environment around their shelter can occur from trees falling, storms or vertebrate movement. These events may change the ability for harvestmen to use the same shelter repeatedly (Hogan *et al.* 2013). However, it is widely known how capable they are of perceiving a wide range of differing environmental stimuli and are dependent on contact chemoreception for information acquisition (Gainerr 2017). Therefore, it would be advantageous for harvestmen to associate environmental chemical stimuli with resources, such as shelter.

I propose to study opiliones associative learning between a chemical stimuli and shelter to validate or invalidate the previous data collected on the subject. This study will increase our understanding of harvestmen learning, since they may have behavioral elasticity and ecological reason to participate in such actions. I hypothesize that harvestmen will associate a shelter with a chemical stimulus. My null hypothesis is, therefore, that harvestmen will not perform associative learning to correlate a chemical stimulus with a shelter.

Methods

I will test if *Discocyrtus invalidus* can use chemoreception to associate shelter with specific chemical surroundings based off the methods of Hogan *et al.* (2013). I will test for associative learning with this species found in forests in Brazil, because it is easy to find, collect, and maintain in captivity. Overall, its natural history allows it to match behavioral flexibility predictions.

Twenty males and twenty female specimens will be collected. This experiment will be performed with different sexes to determine if sex influences my results. The experiment will be conducted in a laboratory setting, so that I can observe behavior and control external influences.

To discern if a specimen associates shelter with a chemical stimuli, specimens will be placed into an arena. A light will be used to motivate specimens to search for a shelter because *D. invidalus* is a nocturnal species and, during the day, rests in a shelter. In Dr. Hogan's experiment, they observed exploratory behavior in specimen before they found the positive stimuli shelter, which seemed to suggest that they had more motivation to explore than to withdrawal from light. This exploratory behavior extended time to find the positive stimuli. I will increase the light intensity previously used to increase discovery time and association with the positive shelter and stimulus, resulting in clearer response data.

An open and a closed shelter (holes in the arena) will be available to harvestmen. The open shelter will be associated with a positive stimuli and the closed shelter (covered with mesh) will be associated with a negative stimuli. Each shelter will be surrounded by filter paper saturated with either Brazilian Rosewood Tree (*Dalbergia nigra*) or Brazilian Timber Tree (*P. echinata*) as the conditioning chemical stimuli. These trees are a part of the natural tropical forest environments that *D. invidalus* inhabits and should be recognizable through contact chemoreception. Additionally, specimens will not eat these stimuli, unlike with food stimuli (Willemart, R. H. *pers comm*). Disks will first be saturated in a container with ground up tree samples (to release tree oils) for 24 hours.

Observation of behavior will be recorded once specimen are placed in the arena. The time it takes to find the open shelter, stimulus first touched, and if both stimuli are touched will be recorded. I will consider an increase in the time to find the open shelter, and changes in the stimulus touched first (negative to positive) as expressions of associative learning. Exploratory behavior will be evaluated as both stimuli being touched and if tugging of the closed shelter mesh occurred.

Each specimen will be individually tested over 28 trials, separated into two rounds of 14 trials. The orientation of the arena will be shifted at random for each trial to provide evidence that an association had been made between the shelter and the chemical stimuli. In the first round of 14 trials per specimen, ten male and ten female specimen will be tested in an arena with the open shelter surrounded with Brazilian Rosewood Tree paper (positive stimuli), and the closed shelter surrounded with Brazilian Timber Tree paper (negative stimuli). Then, I will test the final set of ten males and ten females with the opposite set up. This will account for any preference between the chemical stimuli. In the second round of 14 trials the groups will be subject to an opposite setup to observe if they can learn to associate the opposite stimuli with the open shelter. The order of the individuals and associated sex will be assigned at random for each trial.

A chi-squared analysis will be used to evaluate if the individuals are behaving at random or participating in associative learning. This will be used to investigate exploratory behavior by comparing which trials harvestmen touched both stimuli and which trial they tugged the mesh. It will also be used to investigate learning behavior by comparing the trial number and stimuli first touched. The total time the animal took to reach the shelter for each of the two rounds will be analyzed with an ANOVA using stimulus and sex as between-subject factors and trial as the within-subject factor. A chi-squared analysis will be conducted to compare the two rounds of exploratory behavior to each other, as well as an ANOVA to compare time variance between rounds. I will consider a decrease in exploratory behavior and total time to find the open shelter, as well as this being congruent amongst both rounds of trials as evidence of associative learning.

Expected Outcomes

There are a few possible outcomes to this study. One is that confounding uncontrollable variables could affect the data. In the previous study, they found that time spent before entering the open shelter between trials of individuals did not vary as a function of positive stimulus, sex, or trial (Hogan *et al.* 2013). In this study, I could observe this result; however, in contrast, there could be an influence of these variables on the time to enter the open shelter. I could see an increase or decrease in time as a result of the effect of any of these variables, such as preference for stimuli, influence of sex, or multiple variables influencing behavior in tandem.

Another expected outcome is that my hypothesis is supported and the harvestmen show apparent associative learning. This will be evident if there is a significant change in exploratory behavior, meaning that individuals decreased in the amount of trials spent tugging and interaction with the negative stimulus as trial number increased. I expect to see a significant decrease in time to find the open shelter as trial number increases. This observation, found in Dr.Hogan's study, will suggest that an association between the positive stimuli and open shelter has occurred (2013) and that the negative stimuli has been associated with the closed shelter, therefore, the individuals won't need to investigate such stimulus.

If my hypothesis is supported in the first round, individuals in the second round should be able to form an association between the open shelter and the opposite stimulus that they had previously associated with the closed shelter. I expect that specimen will spend more trials in the second round touching the negative stimulus because they have an association of it with an open shelter. However, specimen will eventually increase their time to find the positive shelter and decrease this exploratory behavior over trials. This shows learning on two levels: 1) that they have learned to associate the first positive stimulus with the positive shelter and 2) they can learn to associate the opposite stimulus with the positive shelter.

My hypothesis will not be supported if the exploratory behavior or the open shelter discovery time do not decrease over trials. This would indicate that the discovery of the open shelter was due to another stimulus or random chance. Rounds may also differ in results because of confounding variables such as captivity time, exhaustion, habituation, etc. on the individuals in the second round. Learning may not be observed or quantifiable if the specimen are experiencing a lack of fitness.

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