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Is the Trail-Following Behavior of Juvenile Physa acuta Influenced by Kinship and Familiarity?

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Abstract

Trail-following behavior is exhibited by many species ranging from insects to gastropods. In gastropods, this behavior serves many functions such as the facilitation of movement, homing, mate and conspecific location, organization, aggregation, and protection. Kin recognition is also a behavior that is exhibited by a wide variety of species including both vertebrate and invertebrate animals. Kin recognition serves many functions including social interactions and status, discrimination, which can lead to outcrossing, and cooperative behaviors that can increase the likelihood of survival. Familiarity with individuals may also contribute to survival through protection and enhanced cooperation. Kin recognition can involve familiarity in conjunction with the detection of genetically-mediated cues or markers, and trail-following mechanisms may be linked to the chemical components of the trail itself. Experiments were conducted to determine whether kinship and/or familiarity influence the trail-following behavior of juvenile *Physa acuta*, a freshwater snail. The degree of trail-following was determined by tracing the trails of marker and tracker snails and by using the lengths of the trails and lines of overlap to calculate a coincidence index. The total length of the tracker trail and marker trail were analyzed to detect differences in marker and tracker trail lengths. There was no significant effect of kinship and familiarity on coincidence index and trail length. There was a pattern for trackers to follow trails of unfamiliar individuals and a pattern whereby tracker snails left longer trails when exposed to the trails of familiar marker snails. Possible benefits for following the trail of an unfamiliar individual may include colonizing a new hospitable environment with other conspecifics or leading the snail away from an inhospitable region. Increase in crawl distance for tracker snails may have been motivated by exploration of new environments, as the presence of a familiar individual would be associated with familiar locations that have already been explored. This study provides insight into the possible role of familiarity in trail-following and locomotive behavior in *Physa acuta*.

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Is the Trail-Following Behavior of Juvenile *Physa acuta* Influenced by Kinship and

Familiarity?

by

Tiana Bonventre

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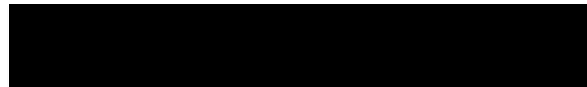
Thesis Committee:

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A THESIS

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TIANA BONVENTRE

Montclair State University

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Introduction

Many animals exhibit the ability to recognize and follow trails laid by themselves and other individuals. This trail-following behavior varies among species and may be driven by multiple factors. These can range from locating prey for lizards and snakes, finding a host for beetles, and nesting for ants and bees (Bonabeau et al., 1997; Cammaerts, Detrain, & Cammaerts, 1990; Furry, Swain, & Chiszar, 1991; Garrett et al., 1996; Jarau et al., 2010). While arthropods such as ants and spiders deposit an invisible pheromone trail (Furey & Riechert, 1999; Sumpter & Beekman, 2003; Tietjen & Rovner, 1980), gastropods use a noticeable mucus layer (Denny, 1989). Pedal mucus has sparked interest as an evolutionary characteristic of motion, especially since excretions of this kind require a great expenditure of energy (Denny, 1980). This led to the notion that trail-following behavior may serve an important function in gastropods (Ng et al., 2013).

Research regarding trail-following behavior in gastropods has revealed that pedal mucus may play an important role in survival and reproduction. Intertidal littorinid snails use trail-following to form aggregates with conspecifics. This can aid in defense against predators and reduce the likelihood of desiccation (Stafford, Davies, & Williams, 2012). In littorinids, following the trail of another individual may also reduce energy expenditure, as the snails produce less mucus when using a previously laid trail (Davies & Blackwell, 2007). Male dioecious gastropods are also able to find and locate females from cues associated with their trails (Erlandson & Kostylev, 1995; Johannesson et al., 2010; Ng et al., 2011). Furthermore, males of some species are able to use trails to distinguish between healthy and unhealthy females. For example, *Littorina littorea* males avoid trails of females that are infected by trematodes that induce female infertility

(Erlandsson & Kostylev, 1995). Mangrove littorinid snails are able to determine which trails were laid by conspecifics and which were laid by heterospecifics, suggesting an adaptive consequence of species recognition and discrimination (Ng et al., 2011; Trott & Dimock, 1978).

Kin recognition is also exhibited by a wide variety of species, and is present among vertebrates and invertebrates alike (Hepper, 1986). Although the mechanisms by which recognition occurs can differ between species, studies have suggested that the recognition adaptation can be linked to a survival benefit, such as increased fitness in choosing unrelated mates (Waldman, 1988). Kin recognition may also be used for social purposes and status, as observed in chimpanzees. Chimps that were given the task of matching mothers with their respective offspring were able to do so with sons and mothers, but had difficulty with daughters and mothers. The authors proposed that in a male dominated society, chimps would benefit more from being aware of male hierarchies and their families (Parr & de Waal, 1999).

Other benefits of kin recognition include the formation of colonies with genetic relatives. Invertebrates such as tunicates can use kin recognition to form colonial fusions, which increase the likelihood of survival and allow for reproduction to occur sooner (Grosberg & Quinn, 1986). Other marine species such as barnacles have also been known to form settlements with kin (Veliz et al., 2006). Even the ability for organisms to discriminate between kin can be favorable. Outcrossing may decrease the likelihood of congenital disorders caused by homozygosity and allow for new genetic combinations (Barrett, 2014). This may also occur in chimpanzees, because females can recognize males that resemble their mothers (Parr & de Waal, 1999).

The behaviors of trail-following and recognition of kin and individuals of the same species can take place through a variety of mechanisms. Kin recognition can occur through physical attributes such as phenotypes, tissue markers such as the major histocompatibility complex (MHC), olfaction, and even previous knowledge or encounters with genetic relatives, also known as familiarity (Hepper, 1986; Waldman, 1988), while trail-following mechanisms may be linked to the chemical components of the trail itself (Ng et al., 2013). Familiarity with other individuals can ultimately benefit the animals involved, since survival behaviors involving foraging and protection have been seen in fish (Barber & Wright, 2001; Ward & Hart, 2003). Guppies are able to learn from familiar individuals where food sources are by shoaling with seasoned conspecifics, as guppies had a penchant for joining groups with more seasoned fish (Swaney et al., 2001). In fish, learning from familiar individuals not only increased the ability to find food, but also enhanced foraging in the presence of potential danger, as group interactions between familiar individuals can lead to more synchronized group swimming (Barber & Wright, 2001). Being familiar with an individual can also reduce aggression. This idea was demonstrated by a decrease in aggression between sticklebacks that were familiar with each other when sharing food (Utne-Palm & Hart, 2000). Decreased aggression also creates better hierarchies among group-dwelling species, promoting order between conspecifics (Barber & Wright, 2001).

Trail-following, kin recognition, and familiarity can contribute to organism survival (Barber & Wright, 2001; Hepper, 1986; Ng et al., 2013; Ward & Hart, 2003). The freshwater snail *Physa acuta* is known to exhibit trail-following behavior (Wells & Buckley, 1972). However, an adaptive benefit for trail-following has not been established

(Ng et al., 2013). *Physa acuta* is a pulmonate snail common to lakes and ponds. In the wild, *P. acuta* have two breeding seasons, one in the spring and another occurring in the fall, with cohorts of this species capable of living up to a year (Maqboul et al., 2014). Freshwater snails feed on aquatic plants and algae, and can be found on submerged vegetation and woody debris (Qazar, 2016; Vasileva, Georgiev, & Gecheva, 2011). *Physa acuta* are also hermaphrodites, allowing them to either outcross or self fertilize (Ohbayashi-Hodoki, Ishihama, & Shimada, 2004). The experiments described in this study were conducted to determine whether kinship and familiarity influence the trail-following behavior of juvenile *P. acuta.*

Methods

Adult *Physa acuta* were collected from Barbour Pond at the Garret Mountain Reservation, Woodland Park, NJ (40°53'55.3" N, 74°10'57.9" W) in October of 2017. Adult snails were maintained in 38 liter aquaria at 23 ± 1°C under a 12:12 light:dark cycle in aerated aged tap water and fed lettuce *ad libitum.* Egg masses were collected as they were deposited, and eggs from the same mass were separated, paired, and placed in 60 ml cups with 6 ml of aged tap water, permitting each subject to have potential kin partners. These snails were fed lettuce and water was changed weekly.

Behavioral trials. Snails with shell lengths ≤ 2 mm were selected for all trials. Trials were carried out in new 60 ml transparent plastic cups containing 4.5 ml of aged tap water. A mark was made on the underside of each cup, designating the center. In each trial, the cup was placed on a clear grid, marked to ensure that the cup remained in the same position and orientation throughout the trial. A marker snail was placed into the cup

on the center point and as the snail moved, its trail was traced with red ink on the grid by

an observer from beneath on a clear plastic sheet for five minutes. The start time was

designated when the snail began to move off the center point. After five minutes, the

marker snail was carefully removed, and the tracker snail was immediately placed on the

center point. The tracker trail was traced as before, but with blue ink, by the observer for

five minutes to determine marker and tracker overlap (Figure 1). The marker and tracker

snail were thereafter returned to the general laboratory population and not used again in

this experiment.

The degree of trail following was determined for each trial by calculating the

coincidence index (CI).

$$CI = \frac{L_c}{L_m \times L_t}$$

where the CI is calculated by dividing the length of the marker and tracker trail overlap

(L_c mm) by the marker trail length (L_m mm) multiplied by the tracker trail length

(L_t mm) (Ng et al., 2013; Townsend, 1974). For a control, marker trails were obtained

from a separate control group (N=20), randomly paired, and superimposed onto one

another. The CI was then calculated for lines of overlap. Trails that were deposited along

the edge of the testing area were not included in the calculations (Methods modified from

Townsend, 1974). The trails and length of coincidence were measured using ImageJ

software (Rueden et al., 2017).

The total length of the tracker trail (L_t mm) and marker trail (L_m mm) were

analyzed for differences in marker and tracker trail lengths. Trails that were deposited

along the edge of the testing area were included in the calculations of total length.

I conducted the experiment using these general methods:

Treatment 1. Unfamiliar-Kin. In this experiment, trials were performed between juvenile snails that were kin (from the same egg mass), but never housed together (unfamiliar). A total of 18 marker/tracker pairs were tested.

Treatment 2. Non-Kin. In this experiment, trials were performed between juvenile snails that were non-kin (from another egg mass), and never housed together (unfamiliar). A total of 18 marker/tracker pairs were tested.

Treatment 3. Familiar-Kin. In this experiment, trials were performed between juvenile snails that were kin (from the same egg mass) and always housed together (familiar). A total of 18 marker/tracker pairs were tested.

Statistical analyses were performed using JMP Pro (v. 13.0) statistical software (SAS Institute, Cary, North Carolina, USA). Coincidence indices between marker and tracker pairs, and total trail lengths of tracker snails, were each analyzed with One-Way Analysis of Variance (ANOVA).

Results

Coincidence Index. There were no significant differences between the treatment groups unfamiliar-kin, familiar-kin, non-kin and control ($F_{3,60} = 0.89$, $P = 0.45$, Figure 2). While the results were not significant, there was a pattern for unfamiliar tracker snails to follow the marker snail trail.

Trail Length. There were no significant differences between treatment groups unfamiliar-kin, familiar-kin, and non-kin in trail length between marker and tracker snails ($F_{2,51} = 1.6$, $P = 0.21$, Figure 3). While the results were not significant, there was a pattern whereby tracker snails left longer trails when exposed to the trails of familiar marker

snails.

Discussion

While the origin of trail production in gastropods is movement, in many species it has evolved to include other adaptive functions that aid in survival (Gould & Vrba, 1982; Ng et al., 2013). These range from homing in limpets (Cook, 1969), food substrate and mate location in littorinids (Davies & Beckwith, 1999; Ng et al., 2011), as well as general organization, aggregation, and protection (Ng et al., 2013; Stafford, Davies, & Williams, 2012). An adaptive benefit for trail-following had not been established in *Physa acuta* (Ng et al., 2013). In the present study, there was no significant difference in coincidence index between treatments and control, although there was a notable pattern in which the snails tended to follow the trail of a marker snail that was unfamiliar. Although this study did not examine adaptive consequences of trail-following, there are possible benefits that might accompany following unfamiliar individuals. For example, following the trail of an unfamiliar conspecific may lead the snail to a new hospitable area or lead the snail away from an inhospitable region the unfamiliar conspecific was leaving. This was demonstrated by other animals such as juvenile lizards that favored establishment in areas containing conspecifics, as dispersal patterns motivated by conspecific cues would ultimately lead to a hospitable environment (Ray & Gilpin, 1991; Stamps, 1987).

Some animal species also exhibit a novelty effect for their environment, objects, and cues in their surroundings (Berlyne, 1950). For example, juvenile rats exhibited a novelty preference for new objects that were introduced to them. Juvenile rats not only had a tendency to advance towards the new objects, but they also explored their

environment more than adults (Stansfield & Kirstein, 2005). It is worth noting that the snails in the present study were also juveniles. As gastropods explore their surroundings, usually with a sensory apparatus such as the tentacles, they may be more likely to detect and follow the novel olfactory and visual cues left by unfamiliar individuals (Ng et al., 2013).

There were no significant differences between treatment groups in trail length between marker and tracker snails. However, there was a pattern whereby tracker snails left longer trails when exposed to the trails of familiar marker snails. Although this study did not examine the function of this behavior, it may have been motivated by exploration of new environments, as the presence of a familiar individual would be associated with familiar locations that have already been explored (Hughes, 1997). Increase in crawl distance for tracker snails may also indicate that the cues from a familiar snail are no longer novel and may not be stimulating to the tracker snail. If so, this could result in a decreased direct response to the familiar snail trail (Thompson & Spencer, 1966) with the tracker snail consequently spending the focal period roaming freely instead of investigating the marker trail cues. This hypothesis is consistent with the pattern of trail-following (higher coincidence index) by unfamiliar snails, but not by familiar snails.

While results of the current study indicate a possible pattern concerning familiarity versus unfamiliarity on trail-following and locomotion in general, there was no evidence of kin recognition or discrimination. Kin selection theory, a form of natural selection that favors heritable variation for helping relatives, allows alleles that cause the bearer to help other bearers of the same allele to become more prevalent (West-Eberhard, 1975). Since closely related individuals have similar genetic alleles, helping relatives

survive can increase the likelihood that those alleles will be transmitted into the next generation (Hepper, 1986). However, there is evidence that kin recognition can collapse (Gardner & West, 2007). When similar organisms associate with each other, individuals bearing shared markers become more prevalent, eventually outnumbering the scarcer ones (Cozier, 1986). Therefore, recognition is lost due to the lack of different markers (Cozier, 1986; Gardner & West, 2007). Furthermore, being familiar with other individuals may supersede relatedness to the animal, since kin are usually the first familiar individuals one would encounter (Ward & Hart, 2003). *Physa acuta* has been known to exhibit inbreeding avoidance through rejection behaviors based on relatedness as adults, particularly during copulation. The snail assuming the female role exhibited shell whipping when the snail assuming the male role was mounting, with increased rejection positively correlated with increased relatedness (Facon, Ravigné, & Goudet, 2006). The authors proposed that in this species, kin recognition may happen during the copulatory act and contact (Facon, Ravigné, & Goudet, 2006).

The composition of pedal mucus and additional information that may be present for the organisms to detect and interpret during trail-following is currently under investigation by other authors, along with other influences on this behavior (Ng et al., 2013).While there is still much to learn about trail-following, more studies on gastropods can give us additional information regarding the evolutionary origins and added functions of trail-following behavior in other species (Ng et al., 2013). This study provides some insight into the possible role of familiarity in trail-following behavior in *Physa acuta*.

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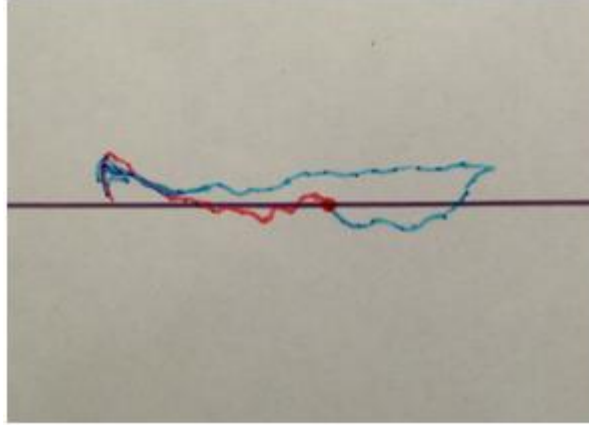
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Figure 1. Example of trails with coincidence (regions in which the trails of the marker and tracker snail overlap).
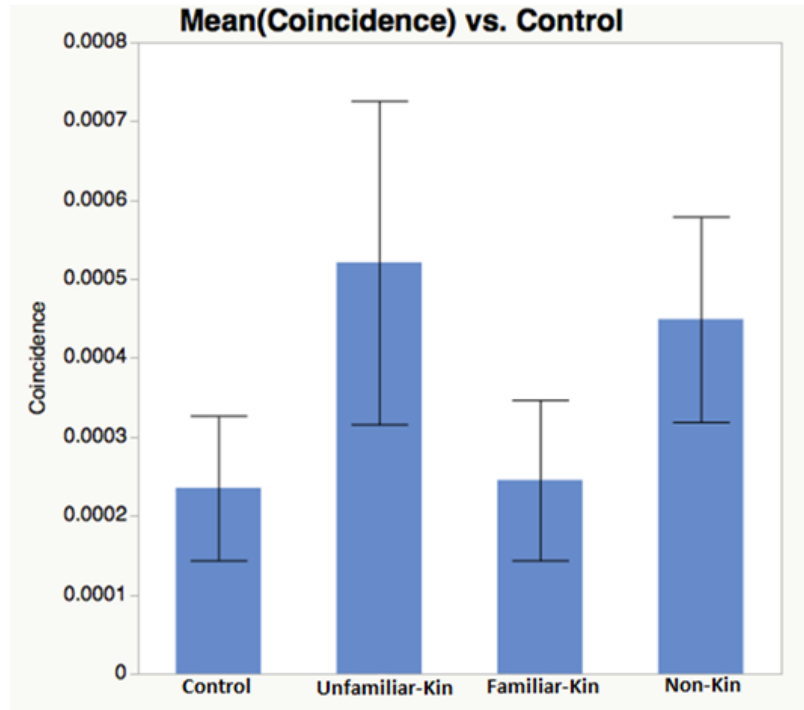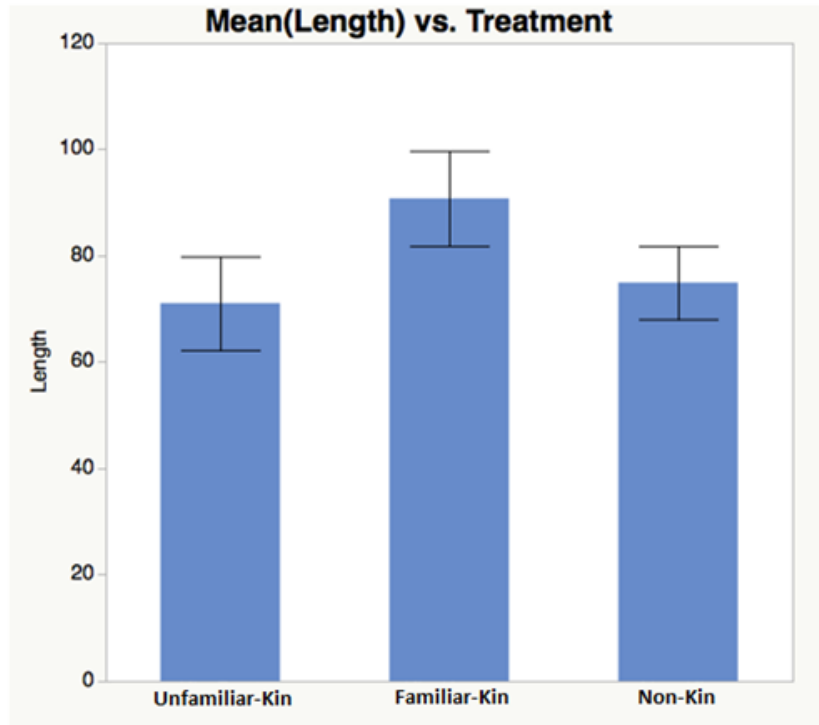
Figure 2. Coincidence index of treatments versus control.

Figure 3. Effect of treatment on trail length.