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Research

Behavior

Differences in Orientation Behavior and Female Attraction by *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in a Homogeneous Resource Patch

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Abstract

The objectives of the study are to understand how naïve beetles disperse after emerging as an adult in a homogeneous resource patch. We compared the movement of adult male and female *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) using a laboratory and a field-collected strain during the first 2 d after their emergence from the wheat kernel in which they developed. We first asked if naïve male and female beetles show any innate orientation pattern. Males showed an upward orientation bias during the first day, but not in the second, whereas females had a random pattern of orientation in both days of evaluation. No significant differences were observed between the two strains. Given that males release an aggregation pheromone, we next asked if the upward movement of males improved their ability to be found by a naïve female. The presence of a male, whether above or below a newly emerged female, changed the females' movement direction from random to bias towards the male. In contrast, free-walking males exhibited the same upward movement bias on the first day regardless of the position of the caged male. Only on the second day did male movement change to the opposite direction of the caged male. Here, we report differences between males and females' movement orientation strategies and their response to males producing aggregation pheromone within the grain mass. Our data may improve our understanding of pheromone attraction and help us to develop better monitoring and control tools.

Key words: pheromone attraction, circular analysis, stored product, insect behavior

Because landscapes have spatiotemporal structure, positive fitness consequences are expected for those individuals that can optimize their movement to find valuable resources (Jander 1975). Natural selection will favor foraging strategies that minimize the organism distance from valuable resources and maximize its distance from stress sources (Doetsch and Gardner 1972, Jander 1975). Moreover, movement patterns can be innate or altered by an organism's experience with the surrounding environment; however, a clear separation of the two is controversial and challenging to determine. One can simply describe innate behavior as a 'stereotyped' or dominant response that is inherited as part of the neuromotor apparatus (Tinbergen 1942). Questions such as what the first impetus of an animal in a new environment is, or how the accumulation of information about the environment can affect future decisions are central to understanding the animal's behavior (Gallistel 1990). Behavioral scientists can test some of those questions by evaluating how a naïve insect behaves in a homogeneous environment under controlled conditions and see how the behavior changes over time. Differences in the innate pattern of movement among individuals could provide insight into differences in orientation strategy between sexes, strains, and even among species (Benhamou and Bovet 1989). Furthermore, if we evaluate movement behavior under experimental conditions similar to those in the original habitat, the results can then be extrapolated to a more realistic scale for application to animal conservation or pest management (Wiens 1977, Wiens and Milne 1989, Ims et al. 1993, Turner 2005).

Bulk-stored grain is a unique ecosystem for insects that exploit seeds and can be a useful model for evaluating the behavioral response of naïve foragers in homogenous landscapes. The ecological dynamics of stored products pests can be divided in within and between patch dynamics as the raw grains, such as wheat and rice, are temporarily stored in large concrete silos or metal bins until processing. In the fine scale, grain is a relatively homogeneous food resource patch that can extend well beyond an insect's short-range walking dispersal abilities (Romero et al. 2008, Cordeiro et al. 2018). On the other hand, at the broader landscape scales, these storage structures are patchy and typically colonized by a group of insects that are adapted to finding and exploiting these resources, eventually traveling from long distances by their flight or by human-mediated dispersal in trains, trucks, and barges (Cordeiro et al. 2019).

Insects that colonize a grain storage bin typically land on the grain surface and move down into the grain mass before feeding and laying eggs (Surtees 1964, 1965). This early stage of the colonization phase is still poorly understood, especially regarding how insects orient themselves when the grain conditions are more homogeneous, and the patch is sparsely populated. As populations increase over time, insects modify the resource patch with their feeding, creating, and responding to gradients in temperature and moisture (Flinn et al. 2011). It is worth noting that pest insects within a grain mass are surrounded by a copious food resource and observed differences in orientation may be related more to finding mates than to finding food (Parker and Macnair 1978).

Rhyzopertha dominica, the lesser grain borer (Coleoptera: Bostrichidae), is one of the most important pests of stored grain worldwide (Potter 2009, Edde 2012). In the United States, when wheat is harvested and stored, it is typically free of infestation but can be quickly colonized by *R. dominica* due to its strong flight ability and abundance in the broader landscape around a grain storage structure (Edde et al. 2005). Eggs are laid on the outside of kernels and first instar chew into a kernel, with all subsequent developmental stages occurring internally until the adults chew out of the grain kernel (Winterbottom 1922, Schwardt 1933, Potter 2009). Upon emerging, males and females need to either disperse to a new resource patch or find each other within the grain mass in which they developed. It has been observed that dispersing individuals from grain storage structures typically have already mated (Ridley et al. 2016, Rafter et al. 2018). *Rhyzopertha dominica* males produce aggregation pheromones (dominicalure-1 and dominiculure-2) when feeding that attract both sexes (Williams et al. 1981). Studies have also shown that both sexes respond by walking or in flight to these aggregation pheromones in similar fashion (Khorramshahi and Burkholder 1981, Williams et al. 1981). Even though evidence for differences in attractiveness can suggest some differences between the sexes as field traps with synthetic lures often capture more females than males (Edde et al. 2005). In addition, pheromone release by males and the measure of the orientation response within a grain patch have not been evaluated yet.

Males and females may behave differently depending on which sex produces the aggregation pheromone. Individuals releasing pheromone are predicted to be less mobile during periods of calling behavior to facilitate location by responding individuals, while non-pheromone releasing individuals in the absence of cues will have more of a ranging search strategy (Ezoe et al. 1994). Prior research with *R. dominica* demonstrated that virgin females in isolation tended to move more and to explore a more extensive area compared to males, and virgin males in isolation were often found closer to the surface of the grain mass than females (Cordeiro et al. 2016). Males being closer to the grain surface than females may help with females locating males in the grain mass given that the pheromone, heavier than air, will tend to move downward in grain patch. Alternatively, the significance of males' position may be more related to the attraction of females to a grain patch or interception of females immigrating into a grain patch. Cordeiro et al. (2016) also found that movement patterns changed with adult age and that patterns of insect feeding damage also influenced movement.

Here, we focused on movement patterns of adults from a laboratory, and a field-collected strain of *R. dominica* immediately after emergence from the kernel in which they developed to determine whether naïve *R. dominica* adults, both males and females, exhibit any directional bias with their movement in a homogenous resource patch. Next, we evaluated how the presence of a calling male, which is the sex that produces the aggregation pheromone, can influence movement orientation within the grain mass by both males and females. Understanding movement mechanisms in resource patches can give us insights on how insects find each other in the absence of directional stimuli and the differences between the sexes in their response to aggregation pheromone.

Material and Methods

Culture Methods

Two different strains of *R. dominica* were used in the experiments. The 'wild' strain was collected outside a rice mill in Otwell, Arkansas, during summer of 2011. Wild strain had been cultured in wheat in the laboratory for only ~10 generations before the experiments were started. The 'laboratory' strain was initially collected in Kansas and had been reared under laboratory conditions for more than 329 generations. During normal culturing, subsamples are collected from a colony jar and transferred to a new jar at regular intervals, which means that this population has not experienced dispersal behavior for more than three decades. Both strains were reared at 28 ± 1°C and 65 ± 5% relative humidity on whole hard red winter wheat, *Triticum aestivum* L., with 13.5 ± 0.3% moisture content. Infested wheat kernels containing pupae were detected using an x-ray image of the grain, separated, and kernel marked with fluorescent pigment powder (Aurora pink DayGlo, Cleveland, OH) to enable easy detection of the source kernel in the bioassay described below. Sex of the beetles emerging from these kernels was determined at the end of the experiment using the methods described by Crombie (1941).

Bioassay

Directional movement of *R. dominica* was measured in a single layer of wheat interspersed between two 40 cm tall by 20 cm wide glass plates (Vardeman et al. 2007, Cordeiro et al. 2016, Cordeiro et al. 2018). Plastic spacers (approximately 1.5 cm wide and 1.1 cm thick) were placed between the two glass plates along the long sides and the bottom. The glass plates and plastic spacers were held together using four binder clips (16 mm capacity), two per long side. This set up provided a gap of approximately 5 mm between the plates of glass, and this gap was filled with approximately 250 g of wheat. The design results in a single layer of wheat between the pieces of glass (i.e., monolayer) that enables insects in the wheat to be visible from at least one of the sides and its position can be tracked by marking on the glass surface with a pen the beetle locations (Cordeiro et al. 2018 for visual representations). The infested wheat kernel identified using x-ray and marked with fluorescent powder was placed in the center of the monolayer. Movement direction and distance on the first and second day after emerging out of the natal kernel into the surrounding grain was measured to address two experimental questions described below.

During experiments, the monolayers were placed vertically inside a box and the top of the box covered with aluminum foil with the tops of the monolayers sticking through the foil. The configuration of the experimental units enabled light only to reach the top of the grain in the monolayers and simulate the environment found in a grain bin. Monolayers were removed from the boxes for observation daily. All observations were made at the same time each day to ensure that movement steps would be captured consistently. However, because of potential variation in time of emergence from a kernel within the first 24 h the actual duration of time captured by the first observation is variable. Preliminary tests demonstrated that earlier or multiple observations per day increased experimental desynchronization and disturbance without significant improvements in parameter collection. The position of the infested kernel placed at the center of the monolayer as well as the two observations of the insect's position was marked on the monolayer glass. At the end of the experiment, the glass plate was photographed using a digital camera. Each image was imported into the image processing software ImageJ and vector angle and movement length calculated for insect positions at 24 and 48 h after emerging from the kernel.

The Orientation of Naïve Beetles in a Homogeneous Environment

An infested kernel from either wild or lab strain was placed at the center of each plate filled with wheat. The plates were observed every day until adult emerged from the kernel and its location was recorded for two consecutive days. From these observations, movement angles and path lengths were calculated. Our null hypothesis was that both males and female movement direction would be random in the homogenous resource patch. Eighteen monolayers were set up at a time and three blocks starting at different time points were completed. Approximately 15 replications for each sex within each strain (lab and wild strains) were tested (total $n = 60$).

The Orientation of Naïve Beetles to an Attractive Stimulus

This experiment was set up like the first, but this time only the field-collected strain was tested. In summary, a caged male *R. dominica* was placed above the infested kernel, in the average location determined in the first experiment, or the opposite direction below the infested kernel. In the first experiment, males tended to move upward so we wanted to evaluate the hypothesis that this might increase the ability of females to find the male. The rationale is simple; males move upward immediately after emerging from the infested kernel, and from a superior position releases the pheromone. Assuming that pheromone is slightly heavier than air, it would settle down under still air conditions in the grain and make it more effective in attracting females. Since both males and females respond to aggregation pheromone, in this scenario we would expect more males and females would move upward toward the male then would move downward toward a male. Male was confined in a rectangular 2 × 3 cm cage made of woven brass wire cloth (~0.6-mm diameter openings) created by folding and sealing three edges with silicon glue leaving an open side. The cage was then filled with clean wheat and a 2-wk-old male inserted through the unsealed edge, and then the open side was sealed with transparent tape. The cage was positioned at the upper corner of the monolayer wholly submerged in the grain mass (~5 cm from the surface, ~5 cm from the left side, ~15 cm from the right side, and ~30 from the bottom) during the monolayer preparation (this corresponds with the average position of males determined in the first experiment). In another setup, we positioned the cage at the lower corner of the monolayer (~30 cm from the surface, ~5 cm from the left side, ~15 cm from the right side, and ~5 cm from the bottom). Fourteen monolayers were set up at a time, randomizing the cage side, and three blocks were completed: approximately 21 replications for each sex were tested under two different conditions (caged male at the top and caged male at the bottom).

Statistical Analysis

Mean vector length (r), a measure of angular dispersion, was calculated using circular statistical procedures (Zar 1996). The value of r varies inversely with the amount of dispersion in the data, and therefore r is considered a measure of concentration. When r assumes the value of zero, the mean vector cannot be estimated, and when it assumes the value of 1, then all the data is concentrated in one direction. Here, we represented displacement as geometric vectors restricted to a bi-dimensional space connecting the initial point to the final point of observation. In the case of the 24 h evaluation, it connected the infested kernel to beetle's first position and for 48 h evaluation connected first observation to second observation. The unit vector and the direction of the resultant vector were calculated as the estimated mean vector (μ_a). The angular variance was calculated by multiplying two by one minus the mean length (r) divided by the sample size of the vector of circular data (Batchelet 1981). Circular uniformity of distribution in each group was tested using the Watson U^2 test for one-sample. For two-way comparison, Wallraff U was used to test mean differences (Cocatre-Zilgien and Delcomyn 1988). All circular analyses were performed using the 'circular' package in R (Agostinelli and Lund 2013). To analyze path length, a GLM model was performed to assess the effect of sex, strain, day after emerging from the kernel, and the presence of caged male position on path length. The degree was used as a measurement of the plane angle in which 1/360 represents a full rotation. Zero degrees indicates that the insect is moving straight to the right, 90° is moving upwards, 180° is moving straight to the left, and 270° is the straight downward movement.

Results

Naïve Beetles' Orientation in a Homogeneous Environment

Wild Strain

Males displayed directional bias during their first movement step (24 h) after emerging from the kernel, while females dispersed uniformly (Fig. 1). The majority of males tested moved upward during the first movement (78.6% had directional trajectory vectors ranging between 0° and 180° and 50% had angle values between 90° and 180°). Male's first movement trajectory was not uniformly distributed according to Watson's Uniformity test ($U^2 = 1.34$; $P < 0.01$) (Fig. 1a). Female first movement step, in contrast, had angular values regularly distributed around the circle (statistically uniform distribution according to Watson's Uniformity test ($U^2 = 0.04$; $P > 0.10$) (Fig. 1a).

By the second movement step (48 h) males no longer exhibited a directional bias and females continued to not have any directional bias: male trajectories and female trajectories (Fig. 1b). Both male ($U^2 = 0.034$; $P > 0.1$) and female ($U^2 = 0.05$; $P > 0.1$) movement trajectories were considered statistically uniform (Fig. 1b).

Lab Strain

Male and female first movement steps showed similar patterns as in the wild strain (Fig. 2). The majority of males tested moved upward during the first movement (66% had directional trajectory vectors between 0° and 180°) and movement was not uniform according to Watson's Uniformity test ($U^2 = 1.35$; $P < 0.01$). Female first movement trajectory was uniformly distributed ($U^2 = 0.13$; $P > 0.10$) (Fig. 2a). By the second movement step, trajectories did not show a directional bias for either males ($U^2 = 0.08$; $P > 0.10$) or females ($U^2 = 0.03$; $P > 0.10$) (Fig. 2b).

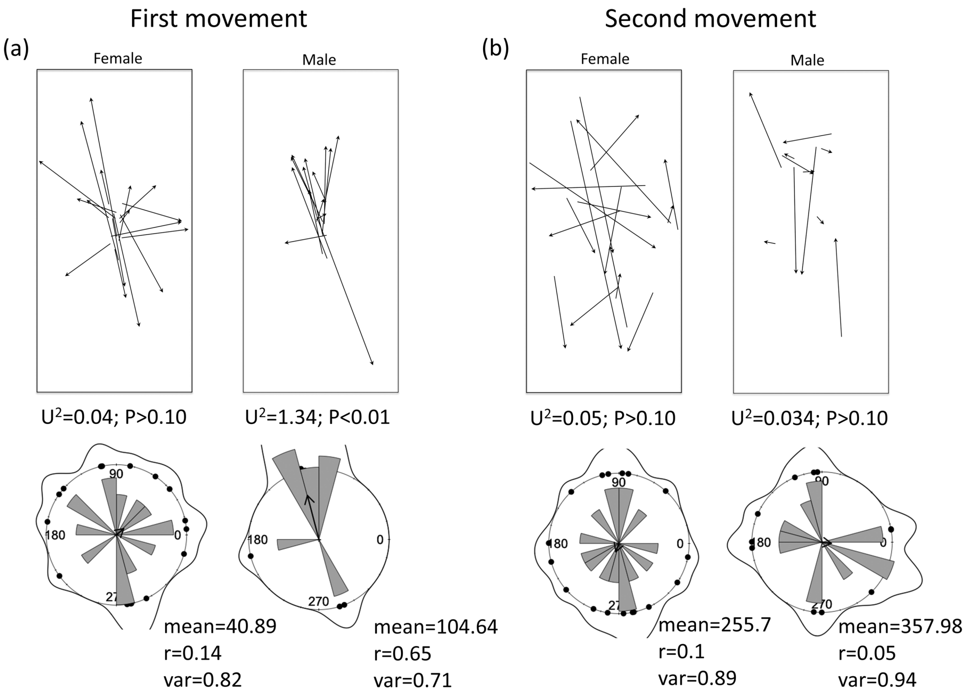

Fig. 1. **Top:** Plots of raw data of females and male from wild strain showing the insect's first movement after insect emerged from the infested kernel (a) and second movement after 48-h evaluation (b). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24-h evaluation (b). **Bottom:** Rose diagram of angle trajectories of *R. dominica* after emerging from the kernel. Arrow direction and size represent the circular mean and the concentration value (*r*). The statistics indicate the mean angle in degree (mean), circular variance (var), the concentration value (*r*), the Watson's Uniformity test to detect directional bias (i.e., *P* < 0.05).

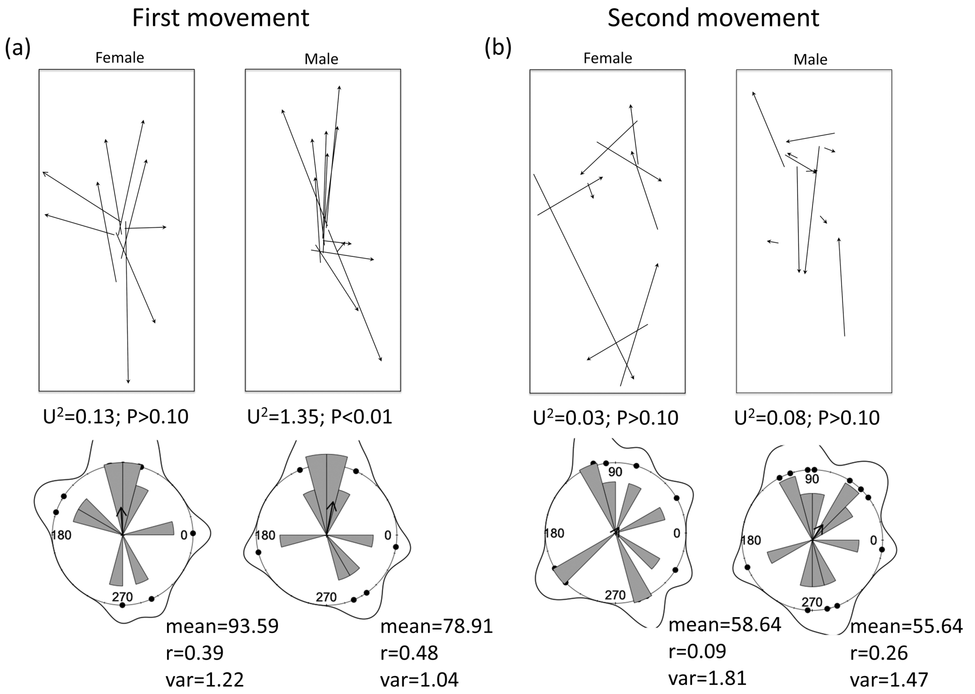

Fig. 2. **Top:** Plots of raw data of females and male from lab strain showing the insect's first movement after insect emerged from the infested kernel (a) and second movement after 48-h evaluation (b). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24-h evaluation (b). **Bottom:** Rose diagram of angle trajectories of *R. dominica* after emerging from the kernel. Arrow direction and size represent the circular mean and the concentration value (*r*). The statistics indicate the mean angle in degree (mean), circular variance (var), the concentration value (*r*), the Watson's Uniformity test to detect directional bias (i.e., *P* < 0.05).

Orientation of Naïve Beetles to an Attractive Stimulus

The presence of a caged male in the top corner of the plate caused a shift in females from nondirectional movement to an upward directional bias ($U^2 = 0.35$; $P < 0.01$) (Fig. 3a). Approximately 83% of females tested had directional vectors ranging from 0° to 180°, with 65% having trajectory vectors between 0° and 90° (cage quadrant) (Fig. 3a). Males also showed a similar biased response in their

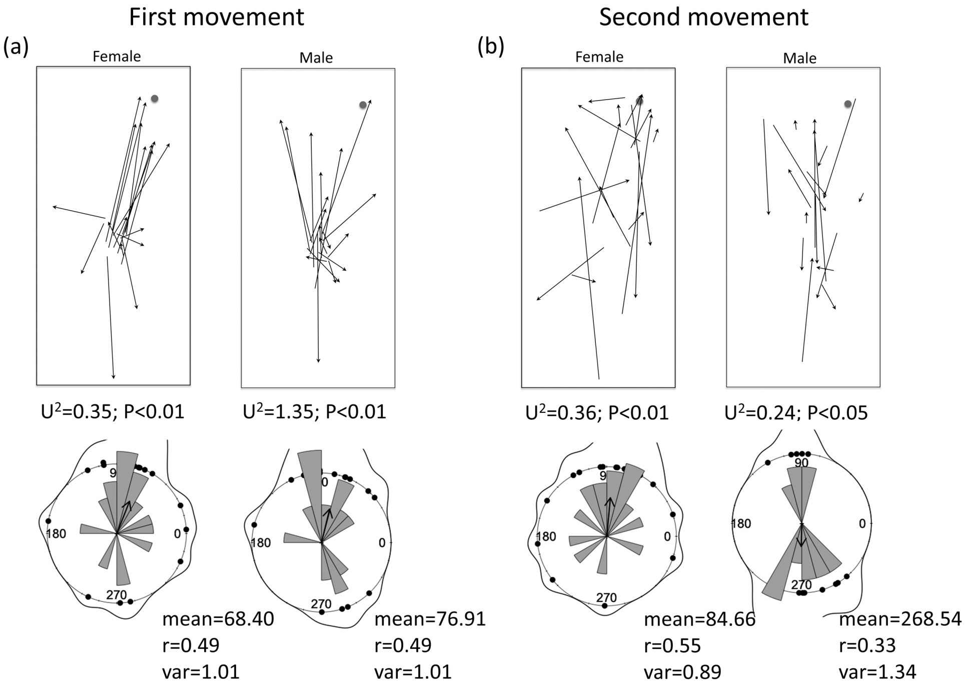

Fig. 3. **Top**: Plots of raw data of females and male from wild strain showing insect's first movement after insect emerged from the infested kernel (a) and second movement after 48-h evaluation (b) in the presence of a caged-male at the top corner of the monolayer (gray circle). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24-h evaluation (b). **Bottom**: Rose diagram of angle trajectories of *R. dominica* after emerging from the kernel. Arrow direction and size represent the circular mean and the concentration value (*r*). The statistics indicate the mean angle in degree (mean), circular variance (var), the concentration value (*r*), the Watson's Uniformity test to detect directional bias (i.e., $P < 0.05$).

first movement after emerged from the kernel (Fig. 3a), and the distribution of movement paths was not significantly uniform in distribution ($U^2 = 0.35$; $P < 0.01$) (Fig. 3a). Although this directional bias was toward the caged male, this was the same direction observed in the controls without a caged male. No statistical difference was detected using Wallraff test for two-sample comparisons ($U = 3.219$, df = 1, $P = 0.07$) between angular mean of the first movement of wild strain males with no caged male and a caged male in the top corner.

The caged male also significantly affected the second movement step of both females and males, with females continuing to move upward toward the caged male, but males switching to moving downward (Fig. 3b). Females continued to have a nonuniform distribution ($U^2 = 0.36$; $P < 0.01$) (82.35% of females' trajectory vectors were between 0° and 180°). Male second movement vector directions had a nonuniform distribution ($U^2 = 0.24$; $P < 0.05$), but in the opposite direction as observed on first 24 h (66.7% of the trajectory vectors between 180° and 360°).

When the caged male was in the bottom corner of the monolayer, the female first movement was also significantly different from nondirectional, but the directionally biased trajectory was downward toward the caged male (Fig. 4). Overall, 94.7% of the female tested had directional trajectories vectors ranging from 180° to 360°, with 79% having trajectory vectors between 270° and 360° (cage quadrant) (Fig. 4a). Female's first trajectory movement was not uniformly distributed according to Watson's uniformity test ($U^2 = 1.04$; $P < 0.01$) (Fig. 4a). Males did not shift from their upward movement when the caged male was placed in the bottom corner and showed a response similar to that observed in the no-cage and cage place at the top corner (Fig. 4b). Movement was significantly nonuniform in distribution ($U^2 = 0.28$; $P < 0.01$) (Fig. 4a). No statistical difference was detected using Wallraff test for two-treatment comparison ($U = 2.56$, df = 1, $P = 0.11$) regarding angular mean of the first movement comparing wild male behavior with no caged male (mean = 104.62; $r = 0.65$; circular variance = 0.71) and caged male in bottom corner (mean = 133.59; $r = 0.43$; circular variance = 1.13).

During the second movement, the female movement vector switched from direction to the bottom corner to uniformly distribute around the circle ($U^2 = 0.07$; $P > 0.1$). However, most of the females remained in the same quadrant as the caged male. The presence of caged male significantly affected the second movement of males. Males' second movement vector directions changed from directional movement to the top left corner to directional movement to the right corner (Fig. 4b). The direction of the second movement of males seem was similar to that of the first movement when no caged male was present: 88% of the direction trajectory vectors between 0° and 180°, with 59% having trajectory vectors between 0° and 90° ($U^2 = 1.34$; $P < 0.01$).

Path Length Analysis

Path length was significantly affected by strain ($\text{mean}_{\text{wild}} = 7.14$ cm, SD = 0.34, $n = 60$; $\text{mean}_{\text{lab}} = 9.40$ cm, SD = 0.88, $n = 42$) and sex ($\text{mean}_{\text{male}} = 7.54$ cm, SD = 7.53, $n = 128$; $\text{mean}_{\text{female}} = 9.06$ cm, SD = 9.06, $n = 125$), but not significantly affected by day ($\text{mean}_{\text{first}} = 8.7$ cm, SD = 0.55, $n = 132$; $\text{mean}_{\text{second}} = 7.85$ cm, SD = 0.57, $n = 121$) or the presence and position of the caged male ($\text{mean}_{\text{no-cage}} = 7.92$ cm, SD = 0.74, $n = 106$; $\text{mean}_{\text{cage-up}} = 8.68$ cm, SD = 0.75, $n = 71$; $\text{mean}_{\text{cage-down}} = 8.27$ cm, SD = 0.73, $n = 76$) (Table 1).

Discussion

In our first question, we asked if naïve beetles would show any orientation biases in a homogeneous environment after having emerged from the wheat kernel in which they developed. We found that males tended to move upward during the first day after emergence from a wheat kernel, but in contrast, females moved randomly during the

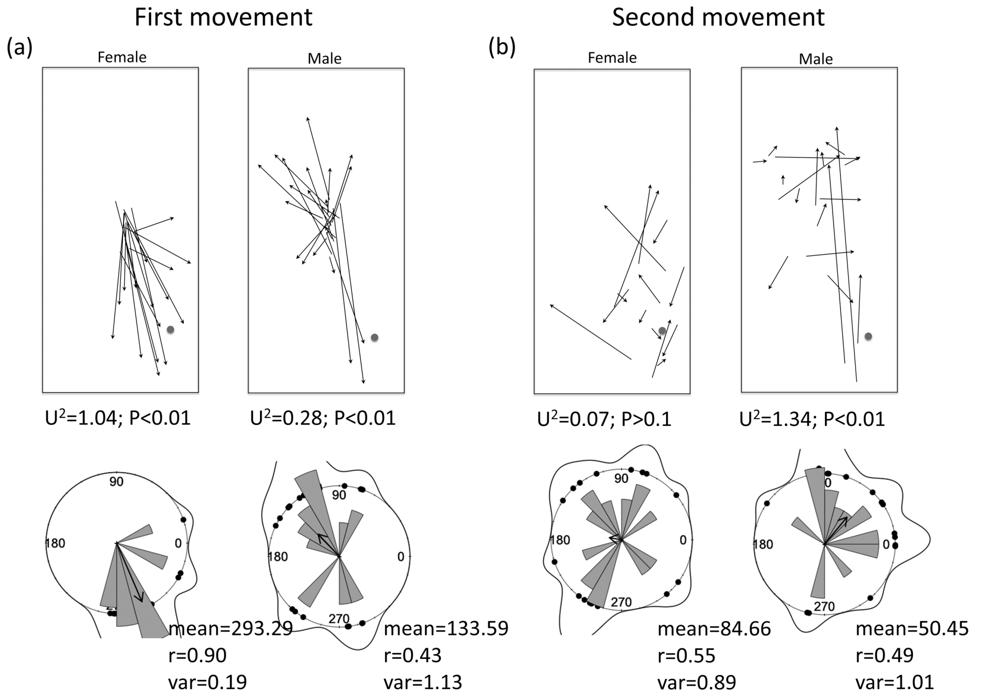

Fig. 4. **Top:** Plots of raw data of females and male from wild strain showing insect's first movement after insect emerged from the infested kernel (a) and second movement after 48-h evaluation (b) in the presence of a caged-male at the bottom corner of the monolayer (gray circle). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24-h evaluation (b). **Bottom:** Rose diagram of angle trajectories of *R. dominica* after emerging from the kernel. Arrow direction and size represent the circular mean and the concentration value (*r*). The statistics indicate the mean angle in degree (mean), circular variance (var), the concentration value (*r*), the Watson's Uniformity test to detect directional bias (i.e., $P < 0.05$).

Table 1. Analysis of variance (type III sums of squares) for four effects on movement path length of the lesser grain borer, *Rhyzopertha dominica* ($n = 126$)

Source of variation	df	MS	*F*	*P*
Sex	1	146.06	6.17	0.01**
Strain	1	145.49	6.15	0.01**
Cage	2	9.66	0.41	0.67
Day	1	44.73	1.89	0.17
Error	247	23.66		

Sex (male and female), stain (wild and lab), cage (no-cage, cage up, cage down), day (24 or 48 h).

**Significant at <0.01 probability.

first day, and both sexes moved randomly in the second day. This pattern was surprisingly similar in both the lab and field-collected strains we tested. This trend could explain the tendency for males to have closer proximity to the grain surface reported using the same type of monolayer assay (Cordeiro et al. 2016). This strong directional bias to male movement is interesting because of it only occurring in the first day of movement when the landscape is most homogeneous. Although *R. dominica* upward movement in grain mass has been reported (Sharangapani and Pingale 1957, Cordeiro et al. 2016), downward movement has been more predominantly observed (Sharangapani and Pingale 1957, Surtees 1965, Keever 1983, Vela-Coiffier et al. 1997, Hagstrum 2001, Mohan and Fields 2002, Vardeman et al. 2007).

In early movement in grain mass experiments, Surtees (1965) reported that most movements occurred within the first 48–72 h of the experiment. In the same study, *R. dominica* was found more frequently in the lower layer of the grain; however, more recent studies have shown that age and crowding can be essential factors not just for the rate of movement (Vardeman et al. 2007), but also for the movement orientation itself (Cordeiro et al. 2016). Our study differs from this earlier research in that we used single insects and allowed them to emerge as adults within the grain mass which can potentially increase the chance of capturing stereotypic behavior pattern without potential behavioral modification through exposure to other individuals and disturbance through handling.

Experiments capturing dispersing females outside of grain storage structures have shown that most have already mated (Edde 2012, Ridley et al. 2016, Rafter et al 2018), which suggests that males and females most likely find each other before dispersing from the grain patch. Females and males are known to mate multiple times during their lifetime, with multiple matings reported to be needed to fertilize all females eggs (Edde 2012, Rafter et al 2018). Males strategy, on the other hand, must be different considering that they are responsible for producing and releasing an aggregation pheromone that attracts both sexes (Khorramshahi and Burkholder 1981, Williams et al. 1981). Males start to release pheromone about 4.7 d after they start feeding (Mayhew and Phillips 1994), and they release pheromone throughout their adult life with a peak around the second week (~12 d old) (Edde and Phillips 2006). Although we focused on the behavior of adults in the first 48-h period after emergence from a wheat kernel, it is unknown how many days of feeding might have occurred after eclosion since both sexes can spend multiple days feeding inside the natal kernel before dispersing. It is unknown what triggers that actual dispersal from the kernel, but it could be linked to physiological maturation so that pheromone could be released, and females are receptive. However, in present work pheromone release was not measured remaining inconclusive to this point. Further research will be needed to determine whether these primary differences in movement are related to pheromone release and could explain differences in movement between males and females.

In our second experiment, we asked if a calling male can influence orientation behavior within the grain mass. Our results show that the presence of a male changed orientation pattern of females. When the male was present, either above or below the female, females had a directional bias toward the male. In contrast, males showed the same directional response upward on the first day regardless of whether another male was present or not, and whether the male was above or below. Those are interesting findings because previous research had indicated that both males and females respond similarly to pheromone. Previous work differed from our study because attraction and response to pheromone were outside of grain patch on clean surfaces with airflow (i.e., using olfactometer Y-shape; Dowdy et al. 1993). It could be that in grain, responses to pheromone are indeed different or that maybe the differences between sexes in response to pheromone only occur during these early periods after emergence from the kernel. Alternatively, our experimental design might not have capture shorter time responses, for instance, responses within the first minutes after emergence from the wheat kernel when the two sexes could have presented a more similar response.

Two possible adaptive hypotheses might explain the pattern of initial upward movement by males. The first hypothesis is that males move upward to improve the ability of females to follow pheromone plume by taking advantage of internal airflow from the headspace or pheromone settling downward under still air conditions. However, while still air conditions are common in grain masses, air movement patterns when they occur are heavily dependent on the inside and outside temperature, which can create seasonal air movement dynamics (Gough 1985, Thorpe et al. 1991). In our test, females were able to effectively respond to the males under still air conditions, whether the males were above or below the point of female emergence. This response does not support the expectation that males on the top could be more easily found. Given the strong response of females regardless of direction, the most likely case is that position in grain does not seem to impact success in terms of female orientation behavior significantly. In our second hypothesis, males move upward to position themselves closer to the grain surface, which could increase the chance of pheromone dispersing from the grain surface and attracting females into the grain patch. However, because we conducted the experiments in a closed system, we were not able to elucidate if males at the grain surface would intercept more females flying from outside. These results raise new questions that need further investigated.

Studies on *R. dominica* orientation and the response pheromone within grain have not been yet conducted, even though this appears to be the environment where mating often occurs. Other studies have shown that at the outside of grain environment, males moved faster in responding to clean grain, but females were more ready to respond when the grain had the odors of *R. dominica* infestation (Nguyen et al. 2008, Bashir et al. 2011). Similarly, according to the latest reports, males spend more time than females in a zone with only grain volatiles, and females spent more time in a zone with pheromone plus grain volatiles (Bashir et al. 2011). Thus, the response of both sexes to pheromone cues might be similar in isolation, but females seem to display a stronger response when grain odors are present (Edde and Phillips 2006, Bashir et al. 2011). The presence of a male in the grain can be potentially significant in *R. dominica* host selection, in particular, affecting the decision of females (Edde and Phillips 2006).

Conclusion

Here, we found that males had a biased orientation behavior after they emerge from the kernel in which they developed even in a very homogeneous environment in terms of temperature, humidity, and grain quality. The function of the stereotypic behavior observed is not known, but it did not improve the ability of females to detect and respond to the male within the grain patch. Females, on the other hand, displayed a random pattern in the absence of the males, but the positive movement towards males potentially producing aggregation pheromone. Males appeared to either not respond or to move away from other males in the time scale evaluated. These results suggest some interesting differences between the sexes in the utilization of aggregation pheromone that has potential impacts on spatial distribution patterns in grain and highlights the need for this aspect of behavior to receive greater attention.

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