PATTERNS OF INFESTATION, DISPERSION, AND GENE FLOW IN RHYZOPERTHA
DOMINICA BASED ON POPULATION GENETICS AND ECOLOGICAL MODELING

by

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B.S., Federal University of Vicosa, 2008
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Abstract

Movement is a fundamental feature of animals that impacts processes across multiple scales in space and time. Due to the heterogeneous and fragmented nature of habitats that make up landscapes, movement is not expected to be random in all instances, and an increase in fitness is an expected consequence for those that can optimize movement to find valuable and scarce recourses. I studied the movement of *Rhyzopertha dominica* (Coleoptera: Bostrichidae), one of the most important pests of stored grain worldwide, within and between resource patches. At a fine spatial scale, I identified factors that contribute to overall and upward movement in the grain mass. Three-week-old insects tented to stay closer to the surface than one or two-week-old insects. Females tended to be more active and to explore more than males. I also found that males tended to stay closer to the surface than females and that might be related to the ability to attract females from outside the patch since there was no significant difference regarding female's attraction within the grain patch. Interaction with feeding sites or other individuals of the same sex creates positive feedback and a more clumped spatial pattern of feeding and foraging behavior. On the other hand, interaction with individuals of different sex creates negative feedback and a more random or overdispersed pattern. At a broad spatial scale, I studied the long-term consequence of *R. dominica* movement on the development of population structure within the U.S. To evaluate population structure, I used reduced representation of the genome followed by direct sequencing of beetles collected from different locations across the U.S where wheat or rice is produced and stored. Ecoregions were more important in explaining structure of *R. dominica* populations than crop type. I also found significant isolation by distance; however, model selection primarily elected grain production and movement variables to explain population differentiation and diversity. Understanding animal movement is essential to

establishing relationships between distribution and surrounding landscape, and this knowledge can improve conservation and management strategies.

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organism's moment and the characteristics of the environment surrounding. This knowledge can improve conservation and management strategies.

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Dedication

I dedicate this dissertation to my loving parents, Elio de Almeida Cordeiro and Maria do Carmo Goes Cordeiro whose words of encouragement and example brought me here. To my beloved brother Eduardo Goes Cordeiro who showed me that "to live is to fight" (*vivere militare est*, Seneca (Epist. 96,5)).

Chapter 1 - Introduction

Animal movement is often considered to be the link between individual behavior and population dynamics (Wein, 2001; Nams, 2006a; Nams, 2006b). For instance, Glanville fritillary (*Melitaea cinxia*) studies demonstrated how individual variation in dispersal propensity can be an important part of the dispersion process and have a direct relation with metapopulation dynamics (Haag, Saastamoinen, Marden, & Hanski, 2005; Hanski & Saccheri, 2006; Niitepõld et al., 2009; Orsini, Corander, Alasentie, & Hanski, 2008; Orsini et al., 2009). Female Glanville fritillary captured in new and more isolated patches had higher mobility than females found in old and poorly connected patches. The variation correlated with a specific allele of a flight metabolic enzyme, which suggests that single genes can lead to profound effects on landscape dynamics and population structure of an organism. Besides intrinsic factors, external factors such as temperature, resource availability, and resource quality are also crucial for understanding movement behavior (Nathan et al., 2008; Turner, 1998). A combination of external factors and the individual sensitivity to those factors will generate a continuum of behavioral states that will eventually lead some individuals to leave their breeding site to colonize a new patch (Bowler & Benton, 2005; Hawkes, 2009). However, separation of movement into movement within a patch and movement between patches (Greenwood & Harvey, 1982) is often didactic for assessment of the relationships between environment conditions, stochastic events, and genetic propensity for dispersing from the breeding site.

Movement within and between patches

Once an animal finds a resource patch, it still has to explore and evaluate the patch (e.g., find areas of higher quality food, locate potential mates, assess quality of nesting sites). This type of foraging behavior is frequently studied at a fine scale by direct observation of the behavior

and testing how 'intrinsic' and 'extrinsic' factors can disrupt behavior patterns (i.e., temperature, resource quality, age, sex, and strain). The way individuals interact with the patch environment and its limits will influence the decision to leave the patch and engage in dispersal movement between patches. The movement between patches can simply be a short distance movement part of the local dynamics, or it can be a long-distance dispersal event, which can have profound ecological and genetic consequences to the populations involved. Unlike the movement within patch, movement between patches happens at a large scale and is difficult to study by direct observation (Nathan, 2001; Nathan, Perry, Cronin, Strand, & Cain, 2003), especially in the case of small animals such as arthropods. One of the difficulties of this kind of study is that the wider the geographic range evaluated the more difficult it is to measure the direct movement between two locations (Slatkin, 1993; Hanski, 1997; Hanski, 1998; Hanski, 1999). However, indirect methods are available to estimate population differences that allow us to infer rate of movement between two distant locations. In my dissertation, I evaluated factors affecting movement within a resource patch by using direct observation of behavior and spatial ecology modeling, and movement between patches by indirect measures of genetic similarity using population genetics tools.

When an organism emerges within a resource patch or when it colonizes a new patch, effective strategies for finding higher quality resources and optimizing resource exploitation can be important adaptations (Able, 1991), since moving exclusively by chance can be energetically expensive and inefficient (Caldwell & Nams, 2005). Therefore, mechanisms and strategies to orient animals towards specific resources in their environment are expected to evolve by natural selection and to show a considerable degree of variation in natural population (Able, 1991). During foraging activities, insects use external sensory information (i.e., visual and chemical

cues) and internally derived information (i.e., variation in reception recognition, movement rate, and learning ability) (Bell, 1983).

Among of the most important sources of external information are chemical cues such as pheromones and kairomones, visual cues such as light intensity, environmental cues such as temperature and relative humidity, sound, and vibration, and these cues may or may not have a directional component (Bell & Kramer, 1979; Bell & Kramer, 1980; Bell, 1983). Non-directional cues do not give away the location of the source of the stimuli, at least not in direct manner, but can cause changes in behavior that lead to more area concentrated search that will keep the organism in the vicinity and increase chance of encounter with source of cues and this behavior is mediated by a giving up time after which, in the absence of reinforcement, the organism resumes ranging search behaviors (Tobin & Bell, 1986; Zimmer-Faust & Case, 1983). Directional movement is a movement towards the information source by moving along the stimulus trail, gradient (i.e. pheromone gradient), visual object (i.e. light or light reflection), or an air or water current (Bell, 1983). For instance, the directional response to pheromones is increased when a wind current is provided, as insects can use the air movement information to go along with the chemical cue (Tobin & Bell, 1986). Likewise, insects can also use different cues to move toward or away from a resource depending on the context or conditions in the immediate environment. For example, precision can be increased when insect relies less on vision and more on olfaction in a dark environment (Hershey & Forester, 1980) or an insect relies less on vision and more on auditory cues to detect stationary prey (Langley, 1983). In a complex environment with confounding stimuli from unknown sources, insect movement is strongly dependent on the way they perceive clues available in the food patch.

The study of how animals move within a resource patch provides useful information on how organisms: make foraging decisions (Bond, 1980; Eccard & Liesenjohann, 2008), use their home range space (Loehle, 1990; Swihart, Slade, & Bergstrom, 2008; Fearer & Stauffer, 2009), determinate scale boundaries and domains (Wiens & Milne, 1989; With 1994a; With 1994b), and ultimately disperse (Weiss & Murphy, 1988; Bowler & Benton, 2005). Animal movement including dispersal behavior is analyzed as a mechanist element of higher ecological processes, and the decision to move is assumed to be a simple response to habitat complexity or a by-product of the landscape pattern. Habitat complexity is often related with environmental heterogeneity and is known to contribute to the observed patchiness in animal distribution and affect how animals move in a landscape (Levin & Segel, 1976; Crist, 1992; McIntyre & Wiens 1999). Elucidating the relationship between landscape pattern and ecological processes is a primary goal of landscape research, and understanding how animals respond to environmental structure – sometimes even creating it – can cast light on many aspects of the nature of movement both within and between patches (Turner, 1989; Turner, 2005). Limitations on spatial and temporal scale force us to often extrapolate results obtained in small-scale experiments to broad scales (Wiens & Milne 1989; Ims, Rolstad, & Wegge, 1993; Turner, 2005).

Long-distance dispersal movement

Unlike movement within a patch where direct observation is feasible and often desirable to assess foraging behavior, evaluating movement between patches has other limitations due to stochasticity, the large spatial scale over which process often needs to be evaluated, and the rareness or seasonality of long-range dispersal event. Dispersal can simply be defined as the movement of individuals away from their source, and divided into short-distance dispersal (SDD) and long-distance dispersal (LDD) (Nathan et al., 2003). The SDD dispersal studies are

important to determine resource usage and small-scale dynamics (Hanski, 1997; Hanski, 1998), whereas the LDD is a highly stochastic, rare, and unidirectional process (Nathan et al., 2003). Unlike movement within the resource patch and SDD, LDD can have an impact at the regional and global scales. LDD can affect spatial spread and colonization rates in which both ecology (metapopulation dynamics) and evolutionary trajectory (gene flow, genetic structure, and species diversity) are influenced (Kot, Lewis, & Van Der Driessche, 1996; Nathan, 2001).

To measure and quantify the LDD of a population can be incredibly challenging (Webster, Marra, Haig, Bensch, & Holmes, 2002). The first problem to be addressed is the proper identification of what is long-range for a particular species. For instance, biogeographical studies of dispersal can potentially involve thousands of kilometers (Briggs, 1995; Nathan, 2001), or a couple hundred meters in the case of metapopulation dynamics within a field crop (Aylor, 1999). Tracking short-distance movement and redistribution of individuals near their source is feasible, but tracking long-distance movement and redistribution of individuals over extended areas is nearly impossible (Webster et al. 2002). Difficulties associated with LDD measurement might cause both the frequency and magnitude of the phenomenon to be under-estimated (Nathan et al., 2003).

With the rapid advance of molecular techniques, genetic methods tend to be more and more common used in the study of LDD, but it also has some disadvantages since genetic methods estimate effective dispersal (i.e., when the individual are able to reproduce in the new location) rather than dispersal *per se* (Nathan et al., 2003). A rich variety of techniques can be applied such as analysis of cytoplasmic structures (i.e., mitochondria), transcripts (i.e., RNA), and genetic markers (i.e., mtDNA and gDNA) to make inferences regarding population differences and similarities. Because genetic markers provide a multilocus estimate on rates of movement,

they have become more popular over the years. The most common genetic analysis methods use patterns of differentiation of alleles within and among populations to estimate dispersal parameters (Slatkin, 1993), and these methods include F_{ST}, coalescence analysis, and modern variations of these methods. Nowadays, high-throughput methods using next generation sequencing (Li, Haipeng, Jakobsson, Sjodin, & Lascoux, 2012; Narum et al., 2013; Reitzel, Herrera, Layden, Martindale, & Shank, 2013) allow us to generate thousands of markers in both coding and non-coding regions across the genome that can be performed in a cost-effective manner to investigate complex questions in ecology (Hohenlohe et al., 2010; Lexer et al., 2014; Benestan et al., 2015; Giska, Babik, von Gestel, van Straalen, & Laskowski, 2015).

Grain stored system and the insect studied

Grain-based products are common in consumer shelves; however, they have to go through several processing, storage and transportation steps before reaching the final consumer (Campbell at al., 2002). From field, to farm bins, to grain elevations, to mills, to warehouses, and to retail stores grain is susceptible to contamination and damage by insects and this can cause a large economic impact on the food industry due to both the direct costs of infestation and the costs due to monitoring and treatment to control pests (Campbell & Arbogast, 2004). Grain storage sites offer suitable condition for insects to grow and to reproduce, and far from being isolated, grain storage sites can be connected by the emigration/immigration and dispersal dynamics of the insects (Toews & Campbell, 2006, Ching'Oma, 2006), but also by human aided movement as grain is moved between storage and processing locations.

The lesser grain borer, *Rhyzopertha dominica*, is one of the most important pests of stored grain in many regions of the world (Potter, 1935). This insect can cause substantial damage by feeding on corn, rice, wheat, and other substrates containing starch (Edde, 2012). *R.*

dominica is an insect with high reproductive potential; females can lay one to seven eggs *per* day externally on the wheat kernels. Egg development takes 12 to 18 days until larvae hatch from the egg. Neonates find and enter individual grain kernels and undergo five instars within the kernel (approximately 30 days). Pupation occurs inside kernel and takes about 6 days at 25°C and when adults emerge they spend a variable amount of time before leave the kernel (Hagstrum & Flinn, 1994; Potter, 1935). *R. dominica* is also considered to be an excellent flier, and can actively cross-infest storage sites in farmlands, flying as far as 3.6 km in a short time interval (Toews & Campbell, 2006, Ching'Oma, 2006). The distribution of this pest is not restricted to a grain storage and processing site, but can potentially extend across multiple storage areas and also into natural areas (Dowdy & McGaughey, 1994, 1998; Hagstrum, 2001). A great number of studies have demonstrated *R. dominica* flight activity with individuals often captured far from grain storage sites, however, the source of this immigration is not well known, nor the potential impact of immigrants from different sources on population dynamics (Edde, 2012).

This species can be found throughout the United States and in all major areas with wheat and rice crops. Due to this great extension of its geographical distribution, it can be found in very diverse environments such as prairies, plains, mountains, valley, highlands, hills, and lowlands (EPA, 1997). These ecoregions – or natural areas – can also potentially play a role in shaping local populations; however, because this species is strongly associated with grain storage sites, the role of the surrounding natural and agricultural landscape on population structure may be more limited than for species not as strongly associated with human created habitats. Because this is a post-harvest pest that infests grain at storage sites, the movement of the grain may also contribute to mixing of populations from far apart locations (Drury, Sinard, & Wade, 2009; Semeao et al., 2012; Nopsa et al. 2015). This phenomenon is generally recognized as responsible

for the lack of population structure previously reported for stored-product pests (Drury et al., 2009; Semeao et al., 2012), although it could also be due to limitations in the markers used to detect structure. Human movement of grain may also create novel patterns of population structure based on patterns of grain production and movement (Nopsa et al. 2015) that may be different than the effects of landscape and distances.

Dissertation outline

The next four chapters of this dissertation explore movement of *R. dominica* adults within the relatively homogenous resource patch of a simulated grain bin, using direct observation of the insect's behavior and manipulating intrinsic and extrinsic variables. Chapter 2, 3, and 4 focus on movement within the resource patch and chapter 5 focuses on the long distance movement between patches. In chapter 2, I evaluated the movement of *R. dominica* within a simulated wheat grain mass trying to identify factors that contribute to overall and upward movement. In chapter 3, I tested if naïve male and female beetles show any innate orientation pattern, and if upward movement of males improved the ability of newly emerged insects to find the male. In chapter 4, I evaluated how pattern emerged within a homogenous landscape by testing beetles' interaction with their environment and with other beetles. In the final chapter, chapter 5, I expanded the scope to evaluate the impact of movement among resource patches and among different regions within the U.S. where grain is grown and stored. I used a genomic approach to estimate differentiation between populations, gene flow, and identified factors that can explain genetic structure in *R. dominica* populations in the United States.

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Chapter 2 - Movement and orientation decision modeling of

Rhyzopertha dominica (Coleoptera: Bostrichidae) in the grain mass

Abstract

Grain stored in bins is initially a relatively homogenous resource patch for stored-product insects, but over time spatial pattern in insect distribution is formed, due in part to insect movement patterns. However, the factors that influence stored-product insect movement patterns in grain are not well understood. This research focused on the movement of the lesser grain borer, *Rhyzopertha dominica,* within a simulated wheat grain mass (vertical monolayer of wheat) and the identification of factors that contribute to overall and upward movement (age since adult emergence from an infested kernel (1, 7, and 14 days), sex, strain, and different levels of environment quality. We also used model selection to select the most relevant factors and determine the relationships among them. Three week-old adults tended to stay closer to the surface compared to one or two week-old insects. Also, females tended to be more active and to explore a larger area compared to males. Explored area and daily displacement were also significantly strain dependent, and increasing grain infestation level decreased daily displacement and explored area. Variation in movement pattern is likely to influence the formation of spatial pattern and affect probability to disperse. Understanding movement behavior within a grain bin is crucial to design better strategies to implement and interpret monitoring programs and to target control tactics.

Introduction

Different types of food grains such as wheat, corn and rice are typically stored after harvest in bulk storage structures such as metal bins, concrete elevators or in bags that are stacked in warehouses. These storage sites represent human-created ecosystems with resource patches that are relatively large and homogeneous, with a relatively stable microclimate, and with negligible flow of energy (Sinha 1995). However, within the broader landscape these resource patches are spatially and temporally patchy in distribution and represent well-defined physical units with limited routes for immigration and emigration. A community of stored-product insects is able to exploit this type of ecosystem (Waongo et al. 2015).

Dispersal is the movement between habitat patches and is different from movement within patches (Greenwood and Harvey 1982). Within-patch movement is associated with finding specific resources such as prey, mates, or oviposition sites. Within-patch movement and tendency to remain within a patch can be influenced by interactions with conspecifics, feedback on patch quality, and encounters with patch edges. Distribution patterns between and within a patch are not typically uniform, with the factors contributing to non-uniform distribution among patches being better understood than the factors contributing to non-uniform distribution within patches (White and Loschiavo 1986, White et al. 1993). Determining what motivates an insect's movement and separating random from non-random movement presents a real challenge to behavioral ecologists. Even if we succeed in identifying all the factors that affect movement behavior, dispersal models may be unfeasible because a large number of parameters need to be included (Turchin 1998).

Measurement of movement of stored-product insects in a grain mass is limited and how spatial pattern in insect distribution is formed has not been connected to variation in individual

behavior (Jian et al. 2004a, 2004b). Although a grain mass is initially relatively homogenous, over time spatial variation in moisture and temperature can be generated and influence spatial distribution of insects (Jian et al. 2003, Flinn and Hagstrum 2011). However, actual measurements of how insects move in grain and how pattern might form in the absence of variation in environmental conditions has not been well studied. Understanding movement behavior of insect in storage is crucial for sampling program development and pest management (Jian et al. 2012).

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)*,* is a species that commonly infests stored wheat soon after it is placed into storage, and its populations can increase in size during the warmer months of the year (Dowdy 1994). *R. dominica* are strong fliers capable of moving through the landscape (Ching'oma 2006, Mahroof et al. 2010), but are generally regarded to not move a lot within a grain patch and as a result are not as readily captured in probe traps placed in grain, compared to other species (Hagstrum et al. 1998, Flinn et al. 2009). Inside the grain mass, *R. dominica* spatial distribution is typically clumped in relative low density and tends towards randomness with increasing adult density (Jian et al. 2012). Another process that is not well understood for stored product insects such as *R. dominica* is emigration from resource patches. There has been research on the flight initiation of *R. dominica* (Perez-Mendonza et al. 1999a, 1999b), but little is known about behavior within a resource patch that might increase emigration and how beetles respond to patch edges. Given the ephemeral nature of the resource patch in time, the process of emigration from patches is critical for population persistence and colonization of new resource patches.

The aim of this study is to investigate how insect intrinsic characteristics such as sex, age, strain, and extrinsic factors such as the decline of resource quality due to population size affect

R. dominica movement patterns within a resource patch and tendency to move to the grain surface (a behavior likely linked with tendency to disperse from resource patch). The surface of the grain represents the largest patch edge and is one of the major routes to leave the grain mass and initiate dispersion. The model selection approach (Thorup et. al. 2006) can be a useful tool to explore a wide range of variables and their interactions that might be associated with insect behavior. Model selection can be used for an exploratory study of strong associations, for instance the covariates of environmental variables with migration decisions (Thorup et. al. 2006). Here, a model selection approach combining all factors contributing to movement variation and orientation decisions was used to identify the most important variables associated with *R. dominica* adult dispersal in a grain mass.

Material and Methods

Wheat and insects

Hard red winter wheat, *Triticum aestivum* L. (13.5 ± 0.3 % moisture content) was used in all experiments. Moisture content was determined using a moisture tester (GAC 2100, Dickey-John Corp, Auburn IL).

Two strains of *R. dominica* were used in experiments. The 'wild' strain was collected outside a rice mill in Arkansas, and had been cultured in the laboratory for 1.5 years before the experiments were started. The 'laboratory' strain was originally collected in Kansas and had been reared under laboratory conditions for more than 30 years. Both strains were reared at 28±1°C and 65±5% relative humidity (R.H.) on whole hard red winter wheat. Sex of beetles was determined using the methods in Crombie (1941), but because this method can be damaging, sex was determined at the end of the experiments. We also measured the wet weight of every insect immediately after each trial.

Monolayer Bioassay

The vertical movement of *R. dominica* was measured in a single layer of wheat sandwiched between two 40 cm tall by 20 cm wide and 3.0 mm thick glass plates (Vardeman et al. 2007). Plexiglas spacers (12 mm wide and 5 mm thick) were placed between the glass plates along the sides and the bottom of the glass plates. This provided a gap of approximately 5 mm between the plates of glass. The glass plates and Plexiglas spacers were held together using four binder clips (16 mm capacity) placed in pairs along the long sides. The space between the glass plates (i.e., monolayer) was filled with ~ 250 g of wheat. This left a gap of 5 cm between the top of the grain and the top of the glass plates.

R. dominica adults were released in the monolayers using one of two methods. In the first method, unsexed one-day-old adults sieved from the colony jars were separated and kept in groups of twenty in 500 g of clean wheat to freely mate and interact with each other until reaching the desired age (7 and 14 days). The insects were released at the center of each monolayer (approximately 18 cm from the top and 9.5 cm from the sides), by inserting a glass tube through the grain (inner diameter of 3 mm and outer diameter of 4 mm) until the tip was at the release point, dropping an adult *R. dominica* down through the straw, and then removing the straw. In the second method, kernels infested with *R. dominica* were placed at the center of the monolayer. Infested kernels containing late stage pupae were detected using an x-ray image of the grain (Guedes et al. 2010). The individual infested kernels were then marked using fluorescent pigment powder Aurora pink (DayGlo[®], Cleveland, OH) before placing in the monolayer to facilitate identification.

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 foil. This

enabled light to only reach the top of the grain in the monolayers and simulate the darkened

environment found in a grain bin. Forty monolayer units were setup at the same time, with each

monolayer representing a single repetition. The monolayers were held in an incubator under

14L:10D (no dawn or dusk), 60% (R.H.) and 28°C.

Monolayers were observed daily, the location of each insect determined, and its position

recorded by placing a mark indicating the observation time on one of the glass plates making up

the monolayer. At the end of the experiment, the beetle positions marked on the glass plates were

digitized by photographing each glass plate and importing each image into image processing

software ImageJ (Abràmoff et al. 2004). The image of the one of the plates was flipped to match

the other plate image for each monolayer unit in order to reverse mirror image. Sequential daily

positions of the beetles were used to estimate daily displacement, total distance moved, and

explored area. Insect 'decisions' to move upward, downward, or to stay were also determined

based on movement of more than one body length upward or downward from the previous

position. *R. dominica* feeding in the monolayer formed distinct zones where visible patches of

flour and frass accumulated. Feeding sites (FS) were evaluated at the end of the experiment by

locating each visible feeding site and in ImageJ enclosing the area of flour and frass with the

smallest possible circle that encompassed the whole feeding site. The area of each circle and

number of circles were then calculated.

Effect of adult age on movement

To assess the effect of adult age on vertical movement patterns in a monolayer, mixed sex

adults of the wild strain were collected within 1 day of emergence from wheat kernels and held

in groups in glass jars containing 500g of wheat until reaching desired age class: 1-day, 7-days,

and 14-days post-emergence. Single adults from one of each age class were added to a monolayer and their position recorded daily for 7-days.

Strain and sex effects on movement

A single infested kernel from either the wild strain or laboratory strain was placed at the center of the monolayer and after the adult emerged from the kernel its position was recorded daily for 28-days. We combined data from both strains to test if sex difference is important regardless of strain origin. Then we tested the effect of strain isolating the effects of sex. The age effect derived from these data was also loaded into the final model by breaking down the data into four time-intervals (1-7, 7-14, 14-21, and 21-28-day interval) that corresponded to those tested in the adult age experiment described above to allow comparison.

Effects of level of infestation (patch quality) on movement

To emulate the degraded environment associated with increasing *R. dominica* density (e.g., damaged kernels, frass material, pheromone) while still enabling an individual beetle to be tracked in the monolayer, infested grain previously frozen to eliminate live insects was mixed in different ratios with uninfested grain. To create infested grain, 5000 adult *R. dominica* were placed in 1.5 kg of wheat for 60 days. At the end of this period, the grain was sieved and insects and fine material accumulated in the jars discarded. Sieved grain was transferred to a sealed container and frozen for over a month to kill all the remaining insects. This contaminated wheat was then mixed with uninfested wheat at four ratios: 0, 1, 10, and 50%.

Statistical analyses

One-way ANOVAs with post-hoc Tukey's HSD tests were used to identify significant variation between levels of the treatments in sample means (n= 20) in a completely randomized

design. The analyses were performed using SAS version 9 software (SAS Institute, Cary, NC). All data are presented as mean ± SEM.

Model selection

In order to select the most important variables to explain insect vertical movement and determine the relationships among those variables a model selection approach based in Akaike Information Criterion (AIC) (Wagenmakers and Farrell 2004) was performed to select the best overall model. We calculated an AIC_c index that takes both descriptive accuracy and parsimony into account, by using delta AIC and Akaike weight (w_i) (Akaike 1973) values for the top 5 candidate models. This statistical method rewards parsimony by penalizing the maximum likelihood for the number of the model parameters (Akaike 1974, Richards 2005). Adjusted R^2 was also calculated as secondary criteria for model selection. General linear models were used to test for significant relationships among the response variables related to the insect's vertical movement in grain mass (area, daily displacement, and distance to the surface) and for insect decision (move "downward", "upward", or "stay") using seven explanatory variables: infestation level of grain (0, 1, 10, and 50%), strain (wild and laboratory), sex (female and male), age (1, 2, or 3 week old insects), mating status (non-mated or mated), number of feeding sites produced (FSN), and area of feeding site produced (FSA). All data analysis for model was undertaken using the statistical application and programming language R using glmulti package (Calcagno and Mazancourt 2013).

Results

Effect of adult age on movement

Different age groups showed significant differences regarding the insect's vertical position and displacement behavior in the grain column (Figure 2.1c). Insects belonging to the

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first age class group (1-7 days old) tended to stay farther from the surface compared to the second (7-14 days old) and the third age group (14-21 days old) (F=4.64; df= 2,56 $P<0.001$) (Figure 2.1a). Daily displacement is an estimate of the rate of movement in the grain mass and was calculated by the average distance displaced *per* day. Insects from the second age group (7-14 days old) had higher rates of displacement compared to the first and the third group evaluated (F=3.31; df=2,56; $P=0.04$) (Figure 2.1b).

Feeding sites produced by the different age groups were also different in number and size (Figure 2.1f). Insects from the second age group produced more feeding sites compared to the first and third age group (F=15.61; df=2,56; $P<0.001$) (Figure 2.1d), while there was no difference in the number of feeding sites produced by the first and third age groups. Insect belonging to the second age group tended to tunnel less at each feeding site, producing smaller feeding sites compared to the other age groups (Figure 2.1e). Feeding site number and average feeding site area had a negative correlation (r=-0.46; $P<0.001$). Adults between one and two weeks in age seemed to be the most mobile and had greatest capacity for dispersion.

Strain and sex effects on movement

Virgin males and females (combined strains) behaved differently in regard to vertical position, movement pattern, and feeding behavior (Figure 2.2). After emerging from inside the wheat kernel, virgin females tended to be found farther from the surface compared to virgin males (F=5.93; df=1,31; $P=0.02$) (Figure 2.2a). Females also had a higher daily displacement rate compared to males (F= 11.7; df=1,31; $P=0.002$) (Figure 2.2b). Explored area, calculated using the area of the polygon formed by the outermost observed points, provided an index of how much of the monolayer was explored by a beetle. Females explored a larger area of the grain monolayer than males (F=30.57; df=1,31; $P< 0.000$) (Figure 2.2c).

The sexes did not differ in the number of feeding sites they created (F=3.51; df=1,31; P=0.069), but males produced larger feeding sites compared to females (F=4.75; df=1,31; P=0.035) (Figures 2.2d and 2.2e). Although virgin males and females showed differences in mobility and feeding behavior, those differences were not converted into differences in body mass (F=2.22; df=1,31; P=0.147) (Figure 2.2f). Because the feeding pattern seems to be different between males and females but the body mass does not differ we also tested if the sum of all feeding site area produced by each beetle would be different regarding sex. Total feeding site area was not significantly different between female (mean=13.1±2.10 cm^2) and males (mean=8.7±2.33cm^2) (F=1.88; df=1,31; P=0.18). Body mass was not significantly correlated with distance to the surface (r=-0.21; n=31; P=0.24), daily displacement (r=-0.09; n=31; P=0.61), explored area (r=0.04; n=31; P=0.81), feeding site number (r=-0.35; n=31; P=0.05), or feeding site area (r=-0.06; n=31; P=0.73).

Regarding strain differences (Figure 2.3), the wild strain tended to spend more time near the surface of the grain than the laboratory strain (F=11.992, df=1,33; P<0.001). The behavior of the sexes did not differ between wild and lab strains (F=3.41; df=1,33; P=0.07): males from both strains tended to stay closer to the surface than females (F=4.07; df=1,33; P=0.04). There was no observed difference in daily displacement between wild and lab strains (F=0.925; df=1,33; P=0.29). However, males and females presented different rates of daily displacement (F=9.17; df=1,33; P=0.004). The lab population tended to explore a larger area compared to the field strain (F= 7.743; df=1,33; P<0.000). Females tended to explore a larger area regardless of the strain (F=50.33; df=1,33; P<0.000).

Effects of level of the infestation (patch quality) on movement

The amount of infested kernels, as an indicator of population density and consequentially patch quality, significantly affected insect position and movement (Figure 2.4). Distance to the surface gradually increased with the increase in ratio of infested grain to uninfested grain ($F=$ 3.21; $df=3,40$; $P=0.03$) (Figure 2.4a); insects exposed to a 50% mixture of infested grain tended to stay farther from the surface compared with insects that experienced a less infested environment. This might have been due to decreased activity. Although daily displacement was not significantly different among densities ($F=2.46$; $df=3,40$; $P=0.08$), the explored area gradually decreased with the increase in the amount of infested grain ($F=4.13$; $df=3,40$; $P=0.02$) (Figure 2.4c). We observed some insect mortality in two treatments; three insects died in the 10% and 4 in the 50% ratios. No insect mortality was observed in any of the earlier experiments, so this suggests that the infested grain may have had some detrimental effects on the beetles. Replicates with insect mortality were not included in the above analysis and additional replicates were performed to replace the missing data.

Model selection

For explored area, the top model elected all predictive variables according to AIC_c criteria (Table 2.1). Mating status, age, sex, and feeding behavior parameters (feeding site number and feeding area size) seem to be consistent predictors and were present in all top five models, but the top model also included density and strain. Comparing density and strain, density was more important in explaining variation in explored area ($w_i=0.28$) than strain ($w_i=0.20$). The adjusted R^2 had low variation between models, from 0.37 to 0.38, and is in accordance to what was found using AIC. Global generalized linear model results show that age ($slope=-7.12$) and density ($slope=-0.4$) are negatively associated with explored area, although, density was not significant at the 0.05 confidence threshold (Table 2.2). Feeding site parameters, feeding site

number (FSN) (*slope*=13.90) and feeding site area (FSA) (*slope*=16.41), were positively associated with explored area (Table 2.2). Analysis of strain and sex is consistent with the previous findings; laboratory strain tended to explore the largest area and females are also associated with larger explored area values than males (Table 2.2).

For daily displacement, density, strain, sex, and feeding site number (FSN) were selected for the top model according to AIC_c criteria. Daily displacement models had the good performance, and showed overall low variation between models, from 0.41 to 0.42, accordance with adjusted R^2 varying (Table 2.1). Feeding site number (*slope*=0.9) was positively correlated with daily displacement. Density was weakly and negatively associated with daily displacement (slope=-0.03), and just significant at 0.1 confidence threshold (Table 2.2). Females were also confirmed to be the sex that showed the highest movement capacity, and the lab strain had the highest values of daily displacement.

The top model for the tendency of a beetle to move toward the surface included density, strain, mating status, and sex. The AIC weight (w_i=0.35) shows that the model is severely penalized when another variable is included (w_i=0.18) or dropped (w_i=0.17). Model performance was the lowest relative to models for explored area and daily displacement according to adjusted R^2. Density, strain, mating status, and sex seem to be the best combination of variables to explain movement to the surface; although adjusted R^2 had no improvement compared to the second best model. More mobile insects tend to maintain overall larger distances to surface, and that behavior is likely due their tendency to explore much more than insects that move closer to the surface. This relation is evident in the correlations between distance to the surface and daily displacement (*r*=0.60; *n*=31; *P*=0.001) and explored area (*r*=0.76; *n*=31; *P*=0.001).

In order to understand what factors affect an insect's decision to move "upward", "downward", or to "stay" at the same level on the grain column, we generated models to elect which variables were most influential to the insect's decision on movement direction. The global models for an insect's decision to move "upward", "downward", or to "stay" include density, strain, sex, and feeding site number (FSN). The same set of variables was selected in the top model of the decision to go "downward" and to "stay" at the same position (Table 2.3). AIC weight for the top model for insect decision to go "downward" was considerably higher compared with the other models (w_i=0.44); the same pattern was observed with the insect's decision to "stay" (w_i=0.32). The models of decision to move "upward" or "stay" had good performance according to adjusted R^2 values ranging from 0.13 to 0.22 for the decision to move "upward" and from 0.32 to 0.32 to decision to "stay" (Table 2.3). Slopes of all parameters elected as best predictors were significant at the P-value level of 0.05 under global generalized linear model results. Although the same parameters are in both "downward" and "stay" top models, the slopes are contrary from one another revealing that the opposite state of moving "downward" is to "stay" (Table 2.4). Only density and sex were present in the top model for decision to move "upward" whereas the difference between the models that included density (w_i= 0.34) and the model that did not include density (w_i= 0.33) was small (Table 2.3).

Discussion

Movement behavior of *R. dominica* was significantly affected by a great number factors tested in this study. Beetles tested during the third week of adult life were observed closer to the surface compared to beetles tested during first and second week. Insects from the second age group clearly represent a transition from the lower half to the upper half of the plate. Insects tested at two weeks of age had higher mobility, which was also associated with a higher count of

feeding sites with smaller average size. Previous studies on flight initiation report that young adults have a greater tendency to initiate flight than older beetles, indicating that age has a strong effect also on flight initiation (Barrer et al. 1993, Aslam et al. 1994, Dowdy 1994). Our findings support the idea that younger insects under certain conditions have higher mobility and might be more likely to engage in dispersal behavior by leaving breeding site although we did not directly test this here. However, since adults in the first week did not tend to move toward the surface there was not an observed linkage between periods of time where beetles tend to move upward and a tendency to initiate flight. There was another difference between the age groups that might have influenced the results: 1-7 days insects were collected soon after emergence and transferred into the monolayer and therefore were less likely to have mated and had less time to interact with conspecifics. *R. dominica* mating behavior occurs within the first 24 h after eclosion (Thompson 1966), so beetles transferred to monolayers on the first day may have a higher probability of having not mated then those in the other age groups. If mating status is associated with differences in movement behavior this might influence the results observed. The two older age groups had respectively one and two weeks of interaction with conspecifics prior to being held in isolation in the monolayer. If this interaction provides information on beetle density, then differences in movement behavior between age groups might be due in part to this difference in experience. Insects in the two-week-age group, with the tendency to move upward, may be those most likely to reach the grain surface and initiate flight; these also may have had sufficient time to mate and possibly lay eggs prior to dispersal. The majority of dispersing females captured in the field are fertilized (Edde 2012).

Males had lower mobility and tended to stay closer to the surface than females. A possible explanation for a sex-biased difference in mobility observed in *R. dominica* might be

that since males produce pheromones that are attractive to both males and females (Khorramshahi and Burkholder 1981) they may have better efficiency calling females from a static position. When isolated, females wandered and explored the patch much more than males. Studies on flight activity under laboratory conditions have shown that males and females are not significantly different in flight initiation (Aslam et al. 1994, Dowdy 1994), even though more females tend to be captured in outdoor traps (Edde et al. 2005). Feeding behavior reveals that males and females seem to feed in different fashion. Males tended to be observed feeding in the same location more frequently and produced larger feeding site areas compared to females. This difference is probably related to males being less mobile than females. Differences in mobility and in feeding behavior had no significant effect on body mass values; however, the sum of all feeding total area produce *per* beetle had no significant difference in comparison between males and females suggesting similar resource consumption. Also, body mass did not differ significantly between males and females and only correlated weakly with other movement parameters. Research on *R. dominica* flight initiation has shown that flight correlates with beetle body mass, with heavier beetles more likely to initiate flight than lighter beetles (Perez-Mendoza et al. 1999, Hagstrum et al. 1999). However, flight initiation is a complex behavior, which can be influenced by interactions with other individuals, which might explain the different results found here with isolated insects in a grain monolayer.

The recently collected wild strain and the strain under long-term laboratory culture differed in their behavior. Females from both strains moved in a similar manner, but males from the wild strain explored a smaller area and tended to stay much closer to the surface compared to males from the lab strain. Distance to the surface and overall level of movement were intrinsically related, showing strong and negative correlation to each other, which can explain the

differences between sexes. Beetles cultured in the laboratory may be under selection due to limited interaction with edges, limited flight opportunities, and higher densities that may influence their dispersal behavior. A tendency to be closer to the grain surface may have adaptive advantages for wild strain males, but be unimportant or disadvantageous to strains confined in laboratory culture jars. For example, males that stay closer to the surface may have an increased chance to attract and mate with recently arrived females that have landed on the grain surface. This male strategy of positioning near the grain surface may not be selected for under laboratory conditions where immigration does not occur. In an earlier study, *R. dominica* was less likely to be collected at the grain surface compared to other stored product insect species, and these beetles did not exhibit any tendency to move towards the surface of the grain mass (Surtees 1963). However, in the current study we tested single insects, and in some cases naïve insects emerging from an infested kernel placed inside the monolayer, and it is possible that movements by these insects could represent expressions of innate behaviors compared to older beetles kept in groups. In a different monolayer experiment, the laboratory strain of *R. dominica* showed a strong tendency to move downward when placed on the grain surface (Vardeman et al. 2007). However, insects may respond differently if placed on the surface of a grain patch that had not previously been exploited, and they might induce colonization behaviors rather than dispersal behaviors. Differences among strains or the impact of laboratory culture on movement in a grain mass has not been previously evaluated, but the impact on flight behavior has been evaluated. *R. dominica* strains collected at southern latitudes flew significantly more than did beetles from northern strains, and F1 hybrid progeny from the crosses of those strains tended to have the maternal phenotype for flight activity, suggesting a genetic basis for *R. dominica* inter-patch movement (Perez-Mendoza et al. 1999b). Wild strains were also more prone to initiate flight

than lab strains, even if lab strain had only been in culture for a few generations (Aslam et al. 1994; Perez-Mendoza et al. 1999a, 1999b).

Decreasing grain quality by overcrowding decreased mobility and increased average distance from the surface, rather than our expectation that low grain quality would trigger avoidance behavior or movement to the surface and ultimately dispersal from the grain patch. Vertical movement studies with other stored grain insect species have demonstrated that insect density increases their diffusion in the grain mass (Jian et al. 2003, 2007; Jian and Jayas 2009). Daily displacement did not differ across the four levels of infestation in our study, but at 1% infestation there was a non-significant trend in the data for movement to be increased. We observed some mortality in the 10% and 50% ratios suggesting that increasing ratio of infested kernels might have a stress effect. An increase of cues associated with infested kernels might produce a toxic environment, and placing beetles in highly infested grain can lead to mortality (JFC, unpublished data). Small changes in behavior due to stress are often difficult to detect due to their non-linear nature, the need to intensively test a narrow range of concentrations, and lack of statistical power (Calabrese and Baldwin 1998). However, despite the reduction of insect mobility and increased average distance from the surface, propensity for flight initiation by *R. dominica* has been reported to increase with increasing beetle density (Barrer et al. 1993, Dowdy 1994, Perez-Mendoza et al. 1999a).

Models generated using the combined data support the univariate study results. Explored area and daily displacement elected similar sets of variables and the relation among variables indicates that young, presumably mated lab strain females under low density conditions were associated with high mobility, whereas older virgin wild strain males under high density conditions were more associated to low mobility. Daily displacement had more parsimony and

elected fewer variables, but had similar relationships to those found in explored area model. Model selection was also able to detect a significant effect of strain that was not clear in the univariate studies. With observational behavioral data sets, adjusted R^2 values in the range of 0.4 can be interpreted as a 'large' effect (Møller and Jennions 2002). The models generated here for 'explored area' and 'daily displacement' were in similar range; however, 'distance to the surface' was much lower (0.09). Movement to the surface was rare event and although they were not able to explain a larger share of the variance, the variables listed in the model were highly significant. The same idea can be applied for the insect 'decision models' that followed similar trend. The models that we generated here are only an approximation of the biological phenomenon being studied, so we can only determine the most influential parameters associated with dispersion and how they are possibly related with each other (Symonds and Moussalli 2011). Further studies combining the significant variables found here can be used to validate the findings reported here and enhance our confidence regarding the factors driving insect movement within resource patches such as grain bins.

Although abiotic factors such as temperature and moisture have been shown to influence *R. dominica* movement behavior and spatial pattern of distribution (Flinn and Hagstrum 2011), we have also found that intrinsic variables such as sex, age, and strain may also through their influence on movement behavior and generate different patterns of spatial distribution. While it is not clear at this point why some of the observed differences might be occurring, according to this research, young females will have a completely different movement pattern compared to old males. Those differences are also affected by strain and the quality of environment surrounding it. The variation found in this study does suggest that intrinsic features of the individual and its environmental interactions are likely to affect the pattern of distribution in the grain mass.

Research on movement and spatial pattern will increase our ability to predict and detect insect infestation through better interpretation of monitoring programs using probe traps, better targeting of treatments, and more accurate models of pest distribution and population dynamics.

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Figures and Tables

Table 2.1 Model selection results for top five models for response variables: area, daily displacement, and distance to the surface and adjusted R^2 values. Best models are those with the lowest AIC value. The Akaike weights can be considered to represent the probability of a model given the data, with weights summing to 1 across candidate models.

Competing models for insect movement	AIC	ΔAIC	w_i *(AIC)*	Adj-R^2
Area				
1. Density, Strain, Mate, Age, Sex, FSN, FSA	2367.9	0	0.29	0.38
2. Density, Mate, Age, Sex, FSN, FSA	2368.0	0.14	0.27	0.38
3. Strain, Mate, Age, Sex, FSN, FSA	2368.6	0.70	0.20	0.38
4. Strain, Mate, Sex, FSN, FSA	2369.5	1.62	0.13	0.38
5. Mate, Age, Sex, FSN, FSA	2369.7	1.80	0.12	0.37
Daily displacement				
1. Density, Strain, Sex, FSN	1146.7	0	0.25	0.42
2. Density, Strain, Mate, Sex, FSN, FSA	1147.0	0.25	0.22	0.42
3. Density, Strain, Mate, Sex, FSN	1147.0	0.25	0.22	0.42
4. Density, Strain, Sex, FSN, FSA	1147.5	0.83	0.17	0.42
5. Density, Strain, Mate, Age, Sex, FSN, FSA	1148.1	1.40	0.13	0.42
Distance to the surface				
1. Density, Strain, Mate, Sex	1418.7	0	0.36	0.09

2. Density, Strain, Mate, Sex, FSA	1420.2	1.46	0.18	0.09
3. Density, Strain, Sex	1420.2	1.49	0.17	0.08
4. Density, Strain, Mate, Age, Sex	1420.2	1.5	0.17	0.09
5. Density, Strain, Mate, Sex, FSN	1420.7	1.98	0.13	0.09

Table 2.2 Global generalized linear model results (predictor variables included including intercept) modeling insect's movement using ecological variables. Weighted parameter estimates and standard errors calculated using parameter Akaike weights (Burnham & Anderson, 2002); Starred parameters are significant at the p-value level of: *** $\leq$ 0.001; **0.01; *0.05.

Selected models for insect movement	Parameter estimate	Standard error	p-value
Area			
Intercept	-52.46	9.74	0.00 ***
Density	-0.40	0.25	0.10
Strain	10. 80	7.41	0.15
Mate	-27.94	9.05	0.00 **
Age	-7.12	3.48	0.04 *
Sex	-50.13	5.86	0.00 ***
FSN	13.90	3.36	0.00 ***
FSA	16.41	5.88	0.01 **
Daily displacement			
Intercept	6.41	0.48	0.00 ***
Density	-0.03	0.02	0.06
Strain	2.32	0.46	0.00 ***
Sex	-4.20	0.40	0.00 ***
FSN	0.92	0.39	0.02 *
Distance to the surface			
Intercept	15.43	2.12	0.00 ***
Density	0.10	0.03	0.00 ***
Strain	2.82	0.03	0.00 ***
Mate	2.82	0.83	0.00 ***
Sex	-2.29	0.71	0.00 **

Table 2.3 Model selection results for top five models for *R. dominica* decision to move down, up, and to stay and adjusted R^2 values. Models represent those with the lowest AIC value. The Akaike weights can be considered to represent the probability of a model given the data, with weights summing to 1 across candidate models.

Competing models for insect decision	AIC	ΔAIC	w_i *(AIC)*	Adj-R^2
Down				
1. Density, Strain, Sex, FSN	-153.6	0	0.44	0.22
2. Density, Strain, Sex, FSN, FSA	-152.0	1.66	0.19	0.22
3. Density, Strain, Age, Sex, FSN	-151.9	1.70	0.19	0.22
4. Density, Strain, Mate, Sex, FSN	-151.8	1.82	0.18	0.22
5. Mate, Age, Sex, FSN, FSA	-136.2	17.41	0.00	0.13
Up				
1. Density, Sex	-161.0	0	0.34	0.15
2. Sex	-161.0	0.08	0.33	0.14
3. Density, Age, Sex	-159.6	1.39	0.17	0.14
4. Density, Sex, FSN	-159.5	1.48	0.16	0.14
5. Sex, FSN	-150.9	10.08	0.00	0.14
Stay				
1. Density, Strain, Sex, FSN	29.2	0	0.33	0.32

2. Density, Strain, Sex, FSA, FSN	30.0	0.84	0.21	0.32
3. Density, Strain, Age, Sex, FSN	30.0	0.86	0.21	0.32
4. Density, Strain, Mate, Sex, FSN	31.0	1.89	0.13	0.31
5. Density, Strain, Age, Sex, FSA, FSN	31.2	2.02	0.12	0.33

Table 2.4 Global generalized linear model results (predictor variables included including intercept) modeling insect's decision using ecological variables. Weighted parameter estimates and standard errors calculated using parameter Akaike weights (Burnham & Anderson, 2002); Starred parameters are significant at the p-value level of: *** $\leq$ 0.001; **0.01; *0.05.

Selected models for insect decision	Parameter estimate	Standard error	p-value
Down			
Intercept	0.12	0.04	0.00 ***
Density	-0.00	0.00	0.01 *
Strain	0.09	0.03	0.00 ***
Sex	-0.12	0.02	0.00 ***
FSN	0.07	0.02	0.001 **
Up			
Intercept	0.26	0.01	0.00 ***
Density	-0.00	0.00	0.15
Sex	0.13	0.02	0.00 ***
Stay			
Intercept	0.31	0.07	0.00 ***
Density	0.00	0.00	0.02 *
Strain	-0.18	0.04	0.00 ***
Sex	-0.26	0.03	0.00 ***
FSN	-0.12	0.03	0.00 ***

Figure 2.1 *R. dominica* adult beetle daily positions and locations of feeding sites in wheat monolayers: (a) mean (+SEM) distance to grain surface for three adult age classes (*F=4.64; df=2,56; P<0.001*); (b) mean (+SEM) daily displacement (cm) of three adult age classes (*F=3.31; df=2,56; P<0.05*); (c) daily observation positions of adults in monolayer over 7 days for the combined 20 individual beetles, with the gray circle showing the insect release location; (d) mean (+SEM) number of feeding sites for three adult age classes (*F=15.61; df=2,56; P<0.001*); (e) mean (+SEM) size of feeding sites per individual for three adult age classes (*F=11.36; df=2,56; P<0.001*); (f) relative position and size of the feeding sites in monolayer after 7 days for the combined 20 individuals; and in Figures 1a-b and 1d-e mean bars with the same letter are not significantly different from each other based on Tukey's Studentized Range *post hoc* test.
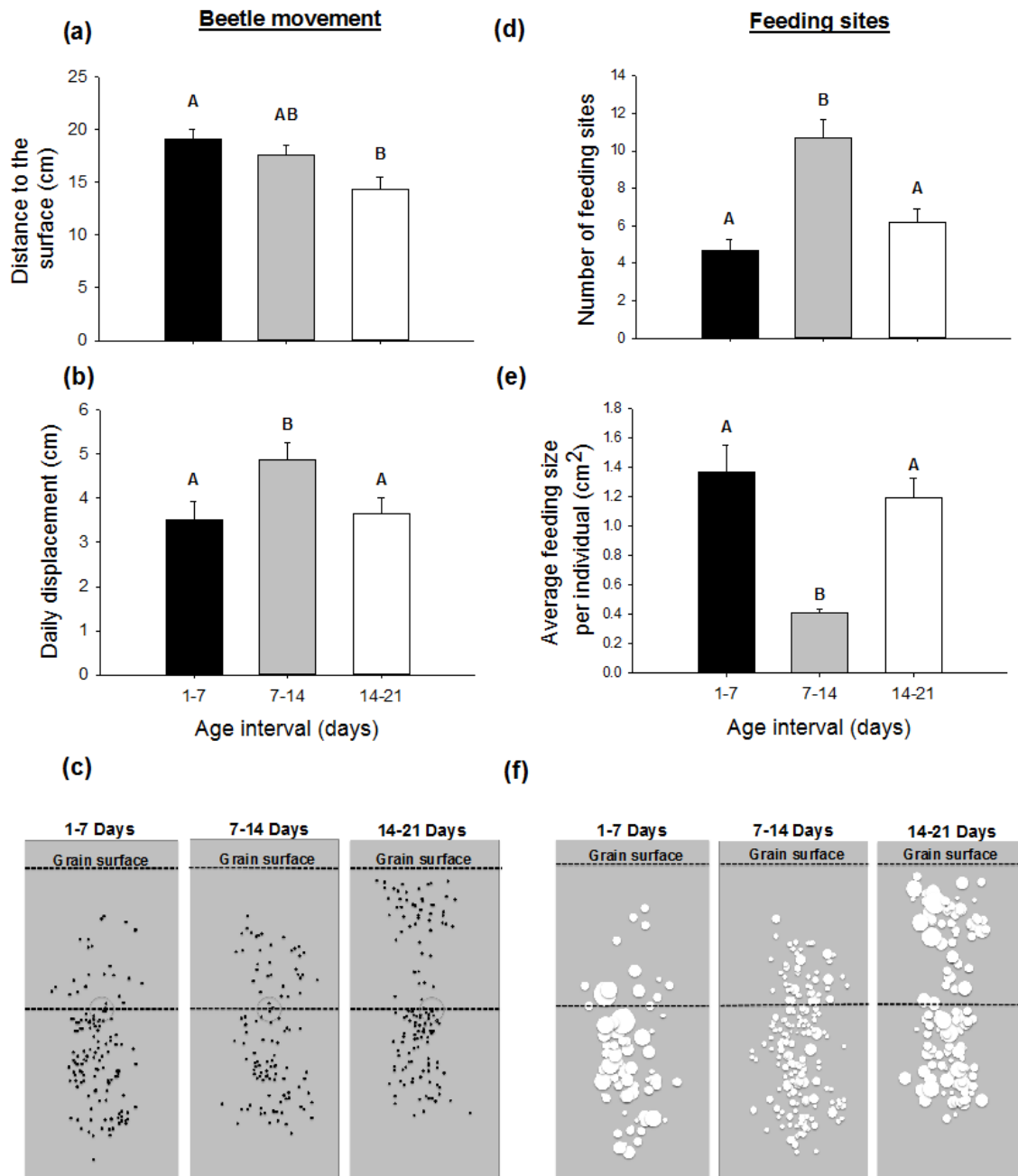
Figure 2.2 Comparison of *R. dominica* female and male movement parameters, feeding site characteristics, and body mass: The box plots indicate the median and dispersion (lower 25% and upper quartiles 75%; bars represent 95% of the observed interval and dots represent data outside this range). (a) Mean (dashed line) of distance to the surface (*F*= 5.93; *df*=1,31; *P*=0.02); (b) daily displacement (*F*=11.7; *df*=1,31; *P*=0.002); (c) explored area (*F*=30.57; *df*=1,31; *P*<0.0001); (d) feeding site number (*F*=3.51; *df*=1,31; *P*<0.07); (e) feeding site average area (*F*=4.75; *df*=1,31; *P*<0.04); and (f) insect's body mass (*F*=2.22; *df*=1,31; *P*<0.15); differences between sexes are indicated using *** for a p-value level of ≤0.001, ** for a p-value level of 0.01, and * for a p-value level of 0.05, and ns indicating a non-significant comparison.
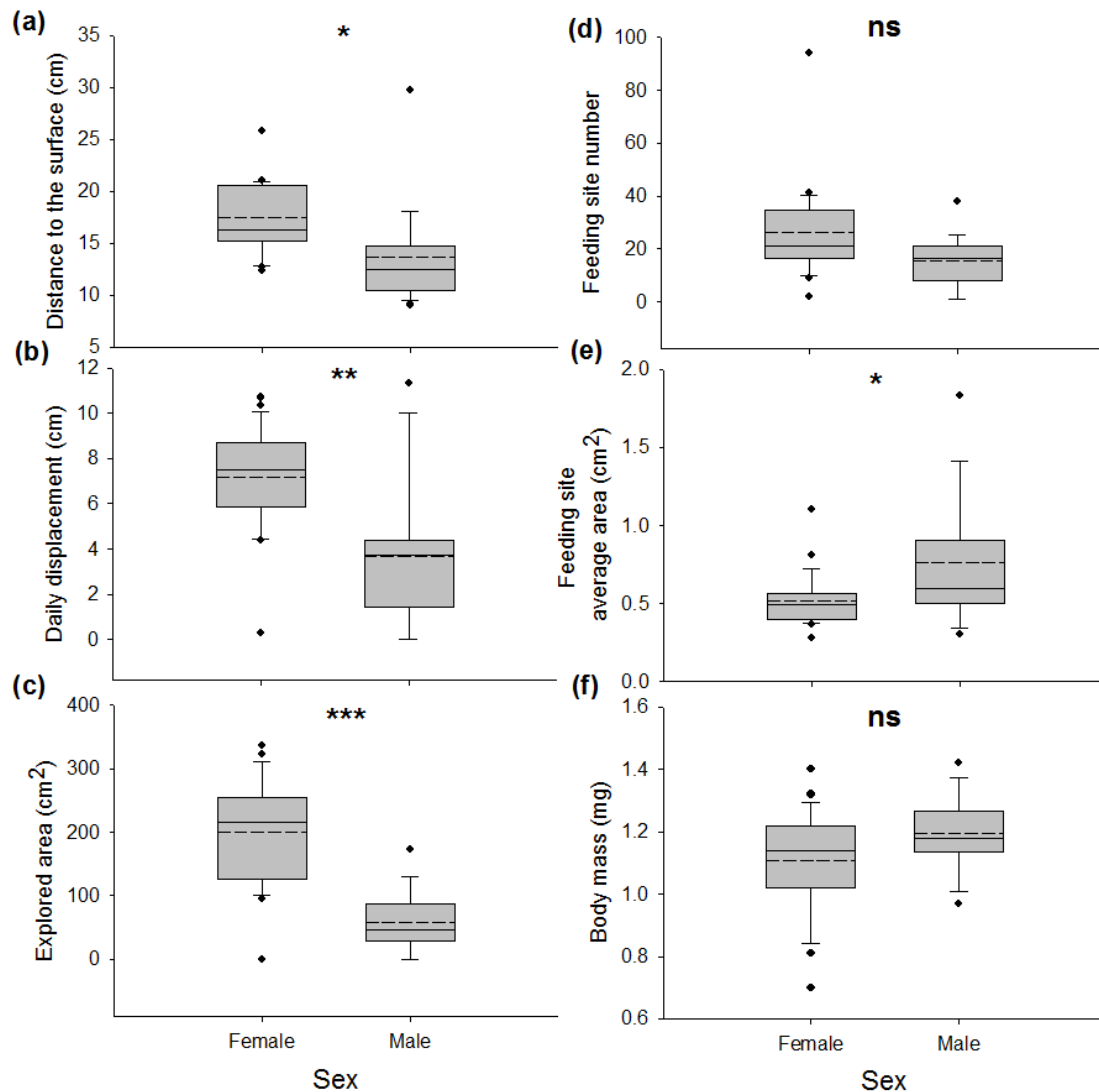
Figure 2.3 Effect of strains on three behavior parameters of insect movement comparing females (gray) and males (white) from wild and laboratory strains: (a) mean (+SEM) distance to the surface (Stain: $F=11.992$; $df=1,33$; $P=0.001$, Sex: $F=1.24$; $df=4,33$; $P=0.36$); (b) mean (+SEM) daily displacement (Strain: $F=0.925$; $df=1,33$; $P=0.29$; Sex: $F=3.8$; $df=4,33$; $P=0.012$), (c) mean (+SEM) explored area (Strain: $F=7.743$; $df=1,33$; $P<0.000$; Sex: ($F=6.5$; $df=4,33$; $P<0.000$).
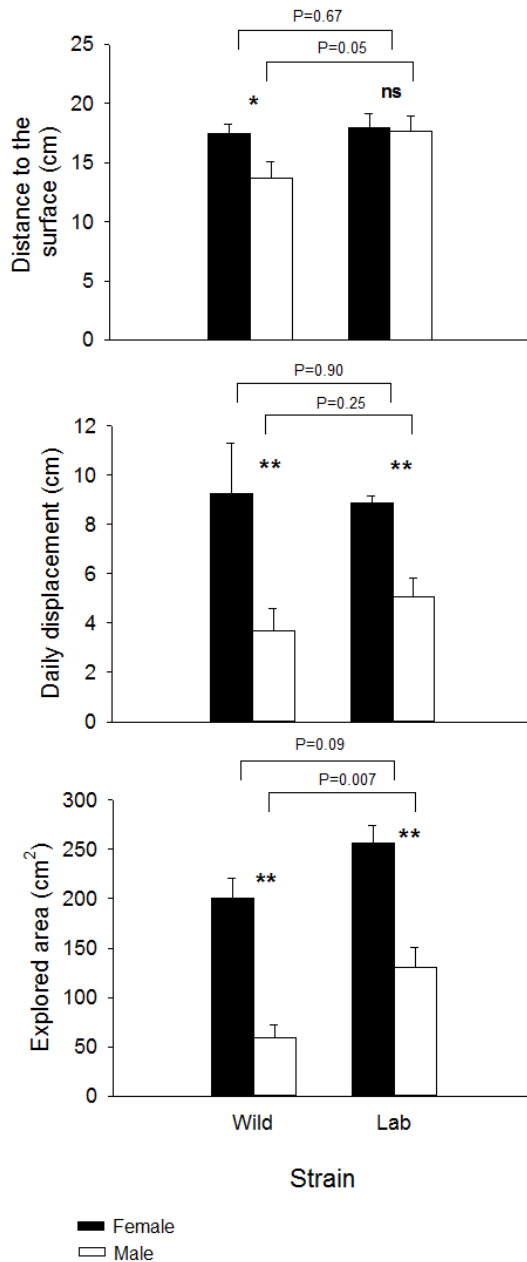
Figure 2.4 Effect of four different grain infestation conditions on three behavior parameters of insect movement comparing three levels of infestation: The box plots indicate the median and dispersion (lower 25% and upper 75% quartiles; bars represent 95% of the observed interval and dots represent data outside this range). (a) Distance to the surface ($F=3.34$; $df=3,40$; $P=0.02$), (b) displacement ($F= 2.46$; $df=3,40$; $P=0.077$), and (c) explored area ($F=4.13$; $df=3,40$; $P=0.0127$) on 0, 1, 10, and 50% of heavily infested kernels mixture with non-infested kernels. Letters represent differences in levels of treatments using Tukey's Studentized Range (HSD) test used for *post hoc* comparison.
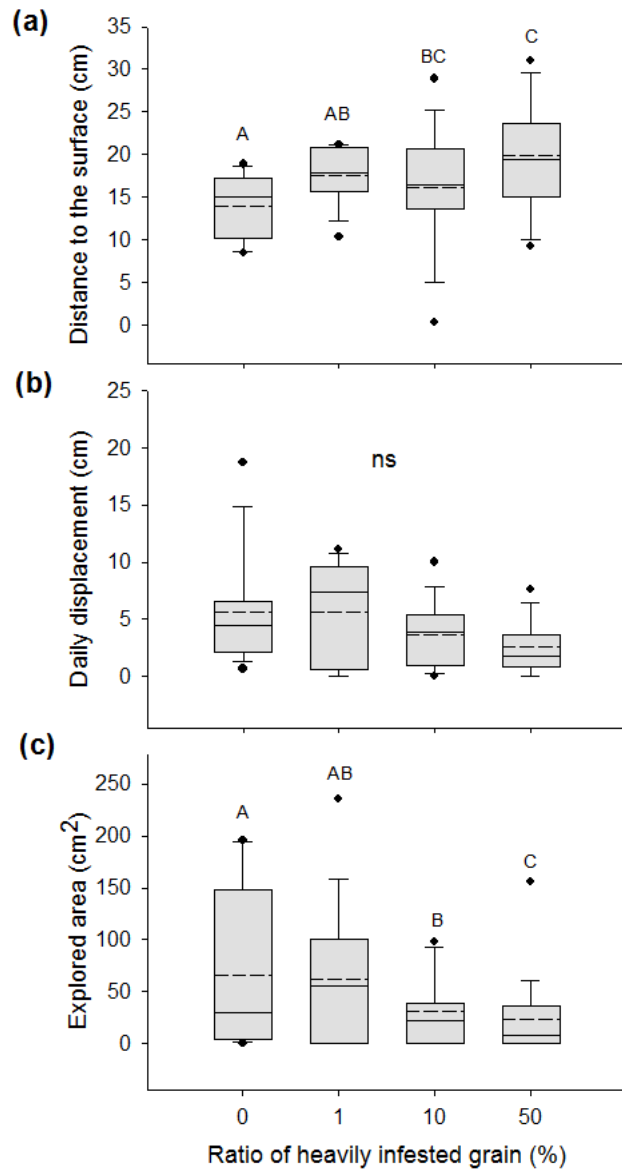
Chapter 3 - Orientation behavior differences and female attraction in a homogenous resource patch

Abstract

The aim of this paper is to determine how naïve beetles disperse after emerging as an adult in a homogeneous resource patch. We tracked male and female *Rhyzopertha dominica* movement, testing two different strains, during the first two days after the adult beetles have emerged 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 of evaluation, but not in the second, whereas females had a random pattern of orientation for both days of evaluation. Similar results were found in both strains. Since males release an aggregation pheromone, we next asked if upward movement of males improved the ability of other newly emerged naïve males or females to find them. The presence of male, whether above or below a newly emerged female, changed the females' movement direction from random to a bias towards the male. In contrast, males exhibited the same upward movement bias on the first day regardless of the position of the male. Only on the second day did male movement change due to the position of the male, with a shift to movement in opposite direction. These results show that upward movement of males during the first day does not improve the ability of other beetles to find them. We observed differences between males and females in their innate movement strategies and response to males producing aggregation pheromone. Based on our findings we hypothesize that upward movement by males may place them in a position closer to the grain patch surface and this could increase interceptions of females arriving to the patch. It is

interesting that outside resource patches both males and females respond to pheromone, but our findings indicate that virgin males within a patch do not respond to other males.

Introduction

Because landscapes have spatiotemporal structure, positive fitness consequences are expected for those individuals that can optimize their movement (e.g., path direction and path length) to find valuable resources (Hutchinson, 1953; Jander, 1975). Orientation fitness is an organism's ability to minimize its distance from resources and maximize its distance from stress sources, and as with any other adaptation is shaped by natural selection (Jander 1975; McIntyre & Wiens, 1999). Innate and learned behaviors can alter orientation and consequently an organism's experience with the surrounding environment; however, a clear separation of the two is not always simple. Evaluation of the first impetus of an animal in a new environment and how the accumulation of information about that environment subsequently changes behavior can be used to evaluate innate and learned behaviors (Gallistel, 1990). Placing a naïve organism into a homogeneous environment can be used to identify innate movement patterns in the absence of cues and then how these behavioral patterns change over time or in response to introduction of heterogeneity can provide insight into the potential fitness benefits of different behavioral strategies and their ecological consequences in terms of spatial distribution. Differences in the pattern of movement among individuals could provide insight into strategy differences between sexes, strains and species (Benhamou & Bovet, 1989). Furthermore, if those pattern of movement are studied in conditions similar to the ones found in the original habitat, the results can be extrapolated to a more realistic scale for application to conservation or management (Wiens & Milne, 1989; Turner, 2005).

Bulk-stored grain is a unique ecosystem for insects that exploit seeds because it is a relatively homogeneous food resource at fine and intermediate scales, since the patch extends well beyond their typical walking dispersal abilities. This food resource also buffers temperature and humidity, which also contributes to homogeneity in conditions. Over time bulk-stored grain can become less homogenous as it is exploited by insects and because of the development of gradients in temperature and moisture due to seasonal or management changes. The grain mass itself is spatially complex for insects that move through the small gaps between seeds and this, combined with limited airflow and low population densities, can create challenging conditions for males and females to find each other (Parker & Macnair, 1978).

Rhyzopertha dominica, the lesser grain borer (Coleoptera: Bostrichidae), is one of the most important pests of stored grain worldwide (Potter, 1935; Edde, 2012). In the United States, when grain is harvested and stored it is typically free of insect infestation, but can be quickly colonized by *R. dominica* due to its strong flight ability and many source populations in the broader landscape (Edde, Phillips, & Toews, 2005). Eggs are laid near kernels and first instar larvae chew into a kernel, with all subsequent developmental stages occurring inside the kernel until newly emerged adults chew out of the grain kernel (Winterbottom, 1922; Schwardt, 1933; Potter 1935). *R. dominica* males produce aggregation pheromones that attract both sexes (Williams, Silverstein, Burkholder, & Khorramshahi, 1981). Attraction to pheromone has been evaluated, with both sexes responding equally (Khorramshahi and Burkholder, 1981; Williams et al., 1981), but the fitness advantages for both sexes responding to the male produced pheromone are not clear for this species. Pheromone lures are used for monitoring flying beetles (Edde, 2012), but response to pheromone has not been previously evaluated within a resource patch such as a grain mass.

Differences between the sexes in the production of pheromone suggests that male and female movement behavior in a resource patch is also likely to be different. 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 pheromone will have more of a ranging search strategy (Ezoe, Iwasa, & Umeda, 1994). Prior research with *R. dominica* has demonstrated that females tended to move more and to explore a wider area compared to males, and males were often found closer to surface of the grain mass than females (Cordeiro, Campbell, & Phillips, 2016). Males being closer to the grain surface than females may help with females locating males in the grain mass since the pheromone, which is heavier than air, will tend to move downward in grain patch. Alternatively, the significance of males' position may be more related to attraction of females to a grain patch and interception of females immigrating into a grain patch.

In addition to what was done previously to investigate the effect of intrinsic (sex and age), and extrinsic (crowding effect) factors on movement behavior (Cordeiro et al. 2016), here we evaluated if naïve *R. dominica* adult males and females of two strains exhibit any directional bias to their movement immediately after emergence from a kernel into a homogenous landscape. Focusing on the differences between sex orientation decision during the first 48h, we want to test if males tented to move upward. Then, since differences in movement were observed between the sexes, we evaluated a potential fitness benefit to the observed movement pattern by evaluating ability of males and females to locate a male that is producing pheromone. Understanding the movement mechanisms in resource patches can give us insights on how insects find each other in a landscape with limited directional stimuli.

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 originally collected in Kansas and has been reared in 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 of any kind for more than three decades (i.e., immigration or emigration population dynamics). Both strains were reared at 28±1°C and 65±5% relative humidity on whole hard red winter wheat, *Triticum aestivum* L., (13.5±0.3 % moisture content). Infested wheat kernels containing pupae were detected using an x-ray image of the grain, separated, and marked with fluorescent pigment powder (Aurora pink (DayGlo®, Cleveland, OH, USA) to enable easy detection of the source kernel in the bioassay described below. Sex of the beetles emerging from these kernels was determined at 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 sandwiched between two 40cm tall by 20cm wide glass plates (Vardeman, Arthur, Nechols, & Campbell, 2007; Cordeiro et al., 2016). Plastic spacers (approximately 1.5cm wide and 1.1cm 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 (16mm capacity). This set up provided a gap of approximately 5mm between the plates of glass and this gap was filled with

approximately 250g 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 either side and its position can be tracked by marking location on the glass surface. The infested wheat kernel identified using x-ray and marked with florescent powder was placed in the center of the monolayer. Movement direction and distance on first and second day after adult emerging into 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. This enabled light to only 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. Observations were made at the same time each day to ensure that movement steps would be captured in a consistent manner, but because of variation in time of emergence from a kernel the actual duration of time captured by the first observation (24 hours) 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 were marked on the monolayer glass. At end of 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 hours after emerging from the kernel.

Naïve beetle orientation 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 daily until adult emerged from the kernel and its

location was recorded once a day (during the afternoon, between 2-5 pm) 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 setup 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).

Male and female naïve beetle orientation to a male releasing pheromone

This experiment was setup like the first, but a caged male *R. dominica* was placed above the infested kernel, in the average location determined in first experiment, or in the opposite direction below the infested kernel. Our hypothesis was that by males moving upward (determined in experiment 1) and then releasing pheromone could be more effective at attracting females since the pheromone being slightly heavier than air may settle downward under still air conditions in the grain. Since both males and females respond to aggregation pheromone, we predicted that both males and females would respond more strongly when caged male is above and less strongly when the caged male is below. To confine the males to a single location, a rectangular 2x3cm cage made of woven brass wire (~0.6 mm diameter openings) cloth was created by folding and sealing three edges with silicon glue leaving an open side. The cage was then filled with clean wheat grain and a two-week old male inserted through the unsealed edge, and then the open side was sealed with transparent tape. Males of this age are predicted to be actively producing pheromone. The cage was positioned at the upper corner of the monolayer completely submerged in the grain mass (~5cm from the surface, ~5cm from the left side and ~15cm 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 other

setup, the cage was positioned at the lower corner of the monolayer (~30cm from the surface, ~5cm from the left side, ~15cm from the right side and ~5cm from the bottom). Fourteen monolayers were setup 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 described in Zar (1996). The value of r varies inversely with the amount of dispersion in the data, and because of that r is considered a measure of concentration. When r assumes the value of zero, the mean vector cannot be estimated and when it assumes 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 initial point to final point of observation. In the case of the 24h evaluation it connected the infested kernel to beetle's first position and for 48h 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 resultant 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 Watson U^2 test for one-sample (Rao, 2006). For two-way comparison, we used Wallraff U test (Cocatre-Zilgien & Delcomyn, 1988). All circular analyses were performed using 'circular' package in R (Agostinelli & Lund, 2013).

To analyse differences in path length, a GLM model with Gaussian family and identity was performed to access the effect of sex, strain, day after emerging from the kernel, and the presence of caged male position. We used degree as a measurement of plane angle in which

1/360 represents a full rotation. Zero degree will be movement straight to the right, 90° is the straight movement upwards, 180° is the straight movement to the left, and 270° is the straight downward movement.

Results

Naïve beetle orientation in a homogeneous environment

Wild strain. Males displayed directional bias during their first movement step (24h) after emerging from the kernel while females dispersed uniformly (Fig. 3.1). The majority of males tested moved upward during the first movement (78.6% had directional trajectory vectors ranging between 0° to 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) (mean=104.62; r=0.65; circular variance=0.71) (Fig. 3.1a) (Watson, 1961). 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) (mean=40.89; r=0.136; circular variance=0.82) (Fig. 3.3b).

By the second movement step (48h) males no longer exhibited a directional bias and females continued to not have any directional bias: male trajectories (mean=357.98; r=0.05; circular variance=0.94) and female trajectories (mean=255.7; r= 0.1; circular variance=0.89) (Fig. 3.1b). 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. 3.1b).

Lab strain. Male and female first movement steps showed similar patterns as in the wild strain (Fig. 3.2). The majority of males tested moved upward during the first movement (66% had directional trajectory vectors between 45° and 135°) (mean=78.91; r=0.48; circular variance=1.04) 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 (mean=93.56; r=0.39; circular variance=1.22) (U^2=0.1262; P>0.10) (Fig. 3.2a). By the second movement step, trajectories did not show a directional bias for either males (U^2=0.08; P>0.10) (mean=55.64; r=0.263; circular variation=1.47) or females (U^2=0.03; P>0.10) (mean=58.62; r=0.09; circular variation=1.81) (Fig. 3.2b).

Male and female naïve beetle orientation to a male releasing pheromone

The presence of a caged male in the superior corner of the plate caused a shift in females from nondirectional movement to an upward directional bias (mean=68.40; r=0.49; circular variance=1.01) (U^2=0.35; P<0.01) (Fig. 3.3a). 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. 3.3a). Males also showed a similar biased response in their first movement after emerged from the kernel (mean=76.32; r=0.49; circular variance=1.01) (Fig. 3.3a), and the distribution of movement paths was not significantly uniform in distribution (U^2=0.35; P<0.01) (Fig. 3.3b). 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 (mean=104.62; r=0.65; circular variance=0.71) and a caged-male in the superior corner (mean=133.59; r=0.43; circular variance=1.13).

Caged male also significantly affected 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. 3.3b). Females continued to have a non-uniform distribution (U^2=0.36; P<0.01) (82.35% of females' trajectory vectors were between 0° and 180°) (mean=84.66; r=0.55; circular

variance=0.89). Male second movement vector directions had a non-uniform distribution (U^2=0.24; P<0.05), but in the opposite direction as observed on first 24h (66.7% of the trajectory vectors between 180° and 360°) (mean=268.54; r=0.33; circular variance=1.34).

When caged male was in the inferior corner of the monolayer, female first movement was also significantly different from non-directional, but the directionally biased trajectory was downward toward the caged male (Fig. 3.4). 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) (mean=293.29; r=0.90; circular variance=0.19) (Fig. 3.3a). Female's first trajectory movement was not uniformly distributed according to Watson's uniformity test (U^2=1.04; P<0.01) (Fig. 3.3a). Males did not shift from their upward movement when caged male was placed in the inferior corner, and showed a response similar to that observed in the no-cage and cage place at the superior corner (mean=133.59; r=0.43; circular variance=1.13) (Fig. 3.4b). Movement was significantly non-uniform in distribution (U^2=0.28; P<0.01) (Fig. 3.4a). 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 inferior corner (mean=133.59; r=0.43; circular variance=1.13).

During the second movement, female movement vector switched from directional to the inferior corner to uniformly distributed around the circle (U^2=0.07; P>0.1) (mean=84.66; r=0.55; circular variance=0.89), which might be due the stronger response within the first 24h compared to when caged male was above. The presence of caged-male significantly affected second movement of males. Males' second movement vector directions changed from directional movement to the superior left corner to directional movement to the right corner (Fig. 3.4b).

Similar to what happened when the cage was in the upper corner, second movement of males seemed to be toward the opposite direction from where the caged male was located: 88.02% of the direction trajectory vectors between 0° and 180°, with 59% having trajectory vectors between 0° and 90° (mean=50.45; r=0.49; circular variance=1.01) (U^2=1.34; $P<0.01$).

Path length analysis

Path length was significantly affected by strain (mean$_{wild}$=7.14 cm, SD=0.34, n=60; mean$_{lab}$=9.40 cm, SD=0.88, n=42) and sex (mean$_{male}$=7.54 cm, SD=7.53, n=128; mean$_{female}$=9.06 cm, SD=9.06, n=125), but not significantly affected by day (mean$_{first}$=8.7 cm, SD=0.55, n=132; mean$_{second}$=7.85 cm, SD=0.57, n=121) or the presence and position of the caged male (mean$_{no\text{-}cage}$=7.92 cm, SD= 0.74, n=106; mean$_{cage\text{-}up}$=8.68 cm, SD=0.75, n=71; mean$_{cage\text{-}down}$=8.27 cm, SD=0.73, n=76) (Table 3.1).

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 for males there was an upward tendency in their movement during the first day after emergence from a wheat kernel, but in contrast females moved randomly during the first day. This pattern was surprisingly similar in the two very distinct strains we tested. This strong directional bias to male movement only appeared during the first day, and by the second day the males moved randomly. This trend could explain the tendency for males to have a closer proximity to the grain surface reported by Cordeiro et al. (2016).

Two possible adaptive hypotheses might explain these results. In the first hypothesis, males move upward to improve the ability of females to follow pheromone plume by taking advantage of the limited inward airflow from the headspace and/or the pheromone settle

downward under still air conditions due to the molecular weight of the pheromone. Generally, there is limited air movement in a grain mass, although when different temperature conditions exist inside and outside a grain storage structure, air convection currents can be generated: air moving downwards at the center and then upwards along the side wall when outside environment is warmer, but the air will move upward at the center and then downwards along the sides when it is colder outside (Gough, Uiso, & Stigter, 1990; Thorp, Tapia, & Whitaker, 1991). The movement of females toward males whether they were above or below the male does not support the hypotheses that being above a female improves the ability to follow pheromone plumes. The potential for irregular movement of air within the grain mass may mean that there is less consistency to pheromone movement and even small amounts of air flow may be sufficient to allow females to locate males at different locations. The expectation was that if airflow is downward, then we should not see the same level of response when caged male was above or below. However, if the air is still, then how do females detect and follow gradients to male? In our system, the slightly better ability to find males below female position suggests that females have some mechanism to respond to pheromone plumes in the grain mass that is not necessarily related to pheromone sedimentation, although the mechanism at this point is unknown. 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 or that males will be better positioned to intercept females immigrating into the patch even if not responding externally to the pheromone.

Although *R. dominica* upward movement in grain has been reported (Sharangapani & Pingale, 1957), downward movement has been more predominantly observed (Sharangapani & Pingale, 1957; Surtees, 1965; Keever, 1983; Vela-Coifier et al., 1997; Hagstrum 2001, Mohan &

Fields, 2002, Varderman et al. 2007). In early movement in grain mass experiments, Surtees (1965) reported that most of the movement occurred within the first 48-72h 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 important factors not just for the rate of movement (Vardeman et al., 2007), but also for the movement orientation (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 stereotypical behavior pattern without potential behavioral modification through exposure to other individuals and disturbance through handling.

It is important to understand the behavioral differences between males and females and the different strategies adopted to find mates prior to and after colonization events involving dispersion. Experiments using outdoor traps have shown that the majority of dispersing females captured in the field were already mated and can be, at least in theory, considered to be an independent unit of dispersion (Edde, 2012). This information suggests that males and females most likely find each other before dispersing from the grain patch. Although most 'pioneer' females arrive in the new resource patch mated, the female can mate again if she encounters another male (Thompson, 1966; Edde, 2012). Females are known to mate multiple times during the lifetime (Edde, 2012). Males, on the other hand, are responsible for producing and releasing pheromone (Khorramshahi & Burkholder, 1981; Williams et al. 1981), and for that reason we expect differences in the way the sexes perceive and respond to environment clues (Gross, 1996). Males start to release pheromone about 4.7 days after start feeding (Mayhew & Phillips, 1994), and they release pheromone throughout their adult life with a peak around the second week (~12 days old) (Edde and Phillips, 2006). In our experiments, the cages males were of an age where

they are expected to be producing pheromone, and the behavioral responses indicate that they were. Males emerging from kernels in our experiments may not be producing pheromone during the first two days after dispersing from the kernel. However, males stay within or near the kernel where it developed after adult eclosion and feed within the kernel. The amount of time spent after eclosion within the kernel can vary from one day to a week, and as a result males during the first 24 to 48hr after dispersal from kernel may be physiologically older and able to produce pheromone. Thus, the pattern of pheromone release can still explain differences in movement between males and females during the current study and in Cordeiro et al. (2016).

In our second experiment, we asked if male and female beetles showed differences in attraction to a stimulus source of a caged male predicted to be releasing aggregation pheromone within the resource patch. Our results show that the presence of a male changed orientation pattern of females. When 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 male was above or below. This is an interesting finding, since 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 was outside of grain patch on clean surfaces with airflow (i.e., using olfactometer Y-shape; Dowdy, Williams et al., 1981; Howard, Seitz, & McGaughey, 1993).

Our results suggest that a male's presence can potentially also affect spatial distribution of the females in the mass depending where the aggregation pheromone source is positioned. Pattern of the second movement shows that males may be avoiding the proximity of the other male (i.e., moving downwards when caged male was above and upwards when caged male was

below), and females seems to be moving in ways to keep them in proximity of the male (i.e., Fig. 3.4, second movement female). According to our results, aggregation pheromone did not significantly attract males within the grain mass on the first or the second day, but may repel males in the second day. More experiments on a different spatial scale and shorter time interval evaluation are necessary to understand further the role of aggregation pheromone within the grain mass.

Studies on *R. dominica* orientation and the response to food odors and pheromone have not been previously conducted within grain. What is known about the system is that outside of grain, female velocity was lower than that observed in males in response to clean grain, but females were more attracted when the grain had the odors of *R. dominica* infestation (Bashir, Birkinshaw, Hall, & Hodges, 2001; Nguyen & Hodges, 2008). In the same experiments, infested grain odors resulted in changes of both female and male *R. dominica* behavior for the parameters evaluated, yet not necessarily in the same way. In a multiple-choice test, males spent more time than females in the zone with only grain volatiles, and females spent more time in the zone with pheromone plus grain volatiles (Bashir et al., 2001). Thus, grain odors may be modifying the response to pheromone cues, with similar response to pheromone in isolation but stronger response by females to pheromone with grain odors (Bashir et al., 2001; Edde & Phillips, 2006). The presence of a male can be potentially important in *R. dominica* host selection, since *R. dominica* females respond more to host volatiles and pheromone together, while males are more prone to use host volatile alone (Edde & Phillips 2006). Aggregation pheromone can certainly be a useful feature recruiting new colonizers to a sparsely colonized area in exploiting grain there does not seem to be a fitness benefit to being groups. Nonetheless, although both sexes respond

to pheromone in isolation, under more realistic conditions in and around grain the pheromone may function more as a sex pheromone than an aggregation pheromone.

Conclusions

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. This movement was primarily upward and positioned males closer to the grain surface, which may be linked to the fact that males are the sex producing pheromones. The stereotypic behavior observed may be related with increasing attraction by females, but did not improve response by females within the grain patch. Research has shown that females respond more strongly to grain with pheromone than males do, and male movement upward in a grain patch might increase attraction of females into a grain patch. Females, on the other hand, displayed random pattern in absence of the males, but directional nonrandom movement towards males that are potentially producing aggregation pheromone. Males exhibited tendency to move upward, however, males move to the opposite direction of the other male after they encounter the other male at the above position. These findings suggest that aggregation pheromone has very different effects on males and female behavior when within a grain mass compared to effects outside the grain.

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Figures and Tables

Table 3.1. Analysis of Variance (Type III sums of squares) for four effects on movement path length of the lesser grain borer, *Rhyzopertha dominica* (n=126). Sex (male and female), stain (wild and lab), cage (no-cage, cage up, cage down), day (24h or 48h).

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		

Figure 3.1 Top: Combined plot 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 48h 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 24h evaluation (b). Bottom: Rose diagram of angle trajectories of *R. dominica* after emerge from the kernel. Arrow direction and size represent the circular mean and the concentration value (r).

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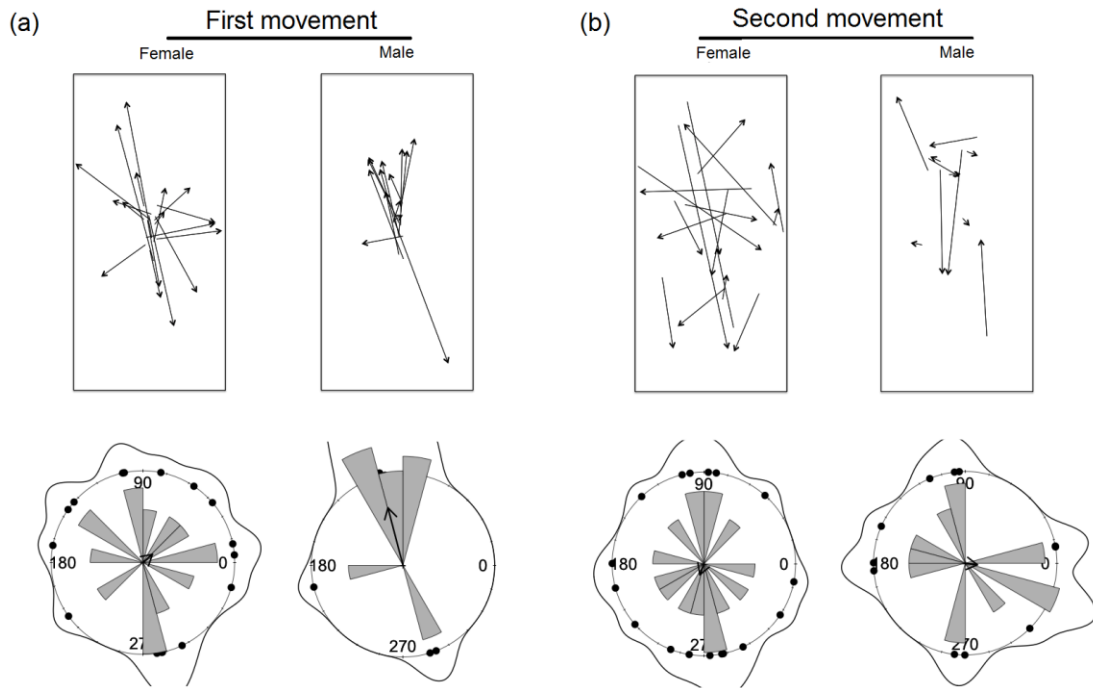
Figure 3.2 Top: Combined plot of raw data of females and male from lab strain showing insect's first movement after insect emerged from the infested kernel (a) and second movement after 48h evaluation (b). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24h evaluation (b). Bottom: Rose diagram of angle trajectories of *R. dominica* after emerge from the kernel. Arrow direction and size represent the circular mean and the concentration value (r).
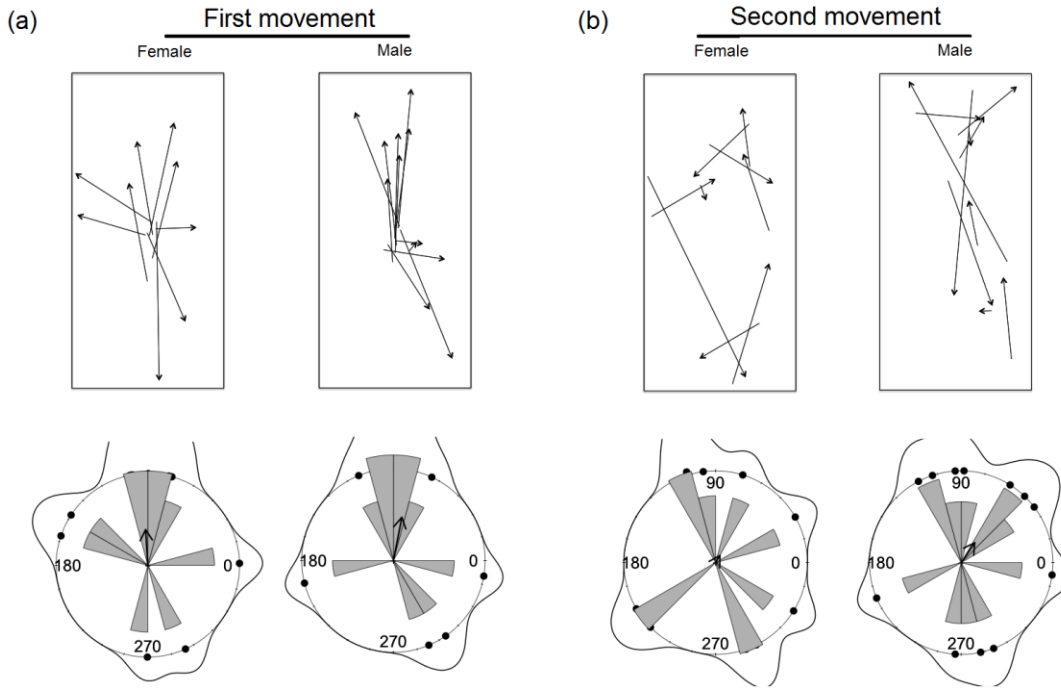
Figure 3.3 Top: Combined plot of raw data of females and male from wild strain showing insect's first movement after insect emerged from the infested kernel (a) and second movement after 48h evaluation (b) in presence of a caged-male at the top corner of the monolayer (gray circle). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24h evaluation (b). Bottom: Rose diagram of angle trajectories of *R. dominica* after emerge from the kernel. Arrow direction and size represent the circular mean and the concentration value (r).
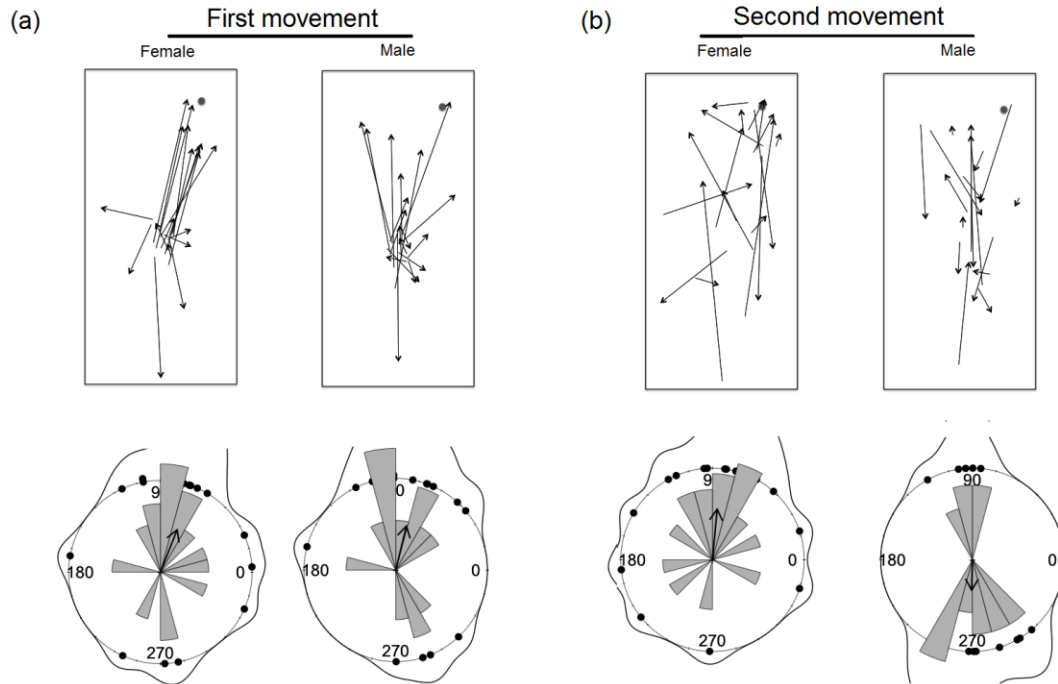
Figure 3.4 Top: Combined plot of raw data of females and male from wild strain showing insect's first movement after insect emerged from the infested kernel (a) and second movement after 48h evaluation (b) in presence of a caged-male at the bottom corner of the monolayer (gray circle). The arrows represent the direction and length of the movement after the insect emerged from the infested kernel (a) and second movement after 24h evaluation (b). Bottom: Rose diagram of angle trajectories of *R. dominica* after emerge from the kernel. Arrow direction and size represent the circular mean and the concentration value (r).
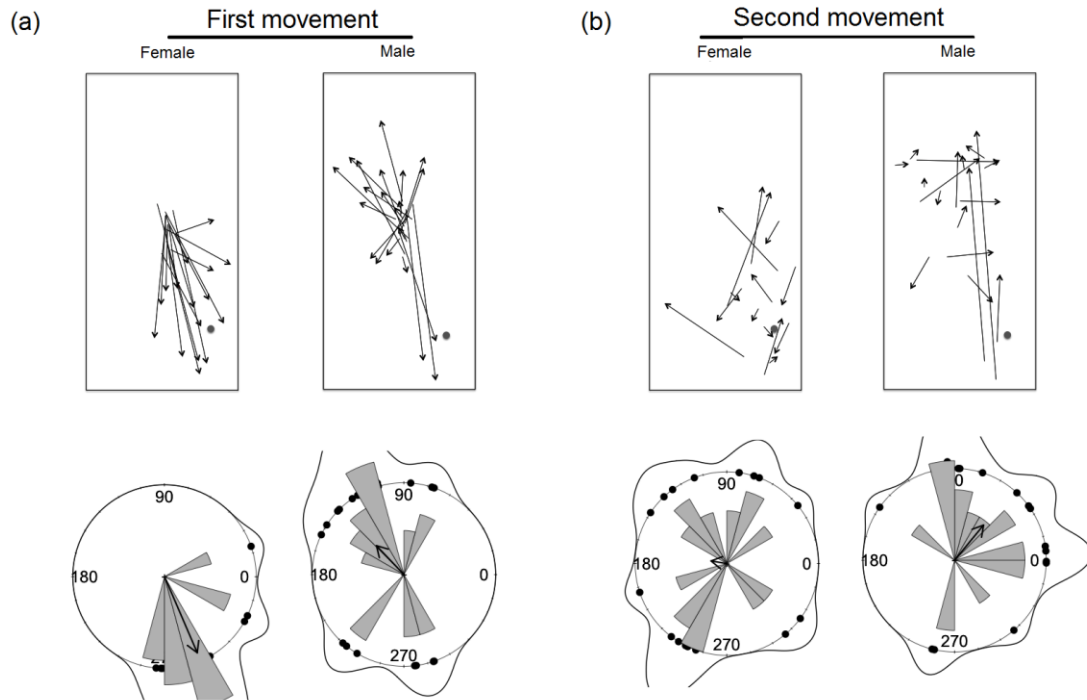
Chapter 4 - Behavioral and ecological mechanisms behind pattern formation: An empirical study of animal movement

Abstract

Environmental heterogeneity influences animal movement patterns and the distribution of individuals in space. In turn, animal movement behavior can create spatial heterogeneity through interactions with the resource landscape and other individuals. To examine the dual role that animal movement behaviors can play in both creating and responding to spatial heterogeneity, we developed an experimental model system consisting of a homogeneous resource landscape (wheat grain), and then evaluated how spatial pattern emerged within these landscapes by studying the movements of the lesser grain borer (*Rhyzopertha dominica*), a seed predator and major pest of stored grain. Our findings suggest the existence of two, non-mutually exclusive sources for pattern formation in this system. First, beetles were significantly attracted to areas where they had previously fed, leading to a positive reinforcement in feeding behavior that ultimately contributed to a patchier resource distribution. Second, beetle movement was influenced by the presence of conspecifics. In the presence of another female, females moved at higher rates (i.e., higher daily displacement) and maintained a more consistent distance from the conspecific, whereas males increased movement rate (daily displacement) and increased path tortuosity with the result being that males and females were found closer together as they moved through the grain. Our results demonstrate that animal feeding behavior; coupled with their response to conspecifics, produce movement patterns that are less random. Our experimental model system thus provides insights into the mechanisms that underlie the reciprocal interaction between behavioral processes and pattern formation in homogeneous landscapes.

Introduction

Understanding how animal movement is influenced by the environment, and how the environment in turn affects the spatial distribution of individuals, has long been a research focus of ecologists and has important implications for the management and conservation of populations. Movement patterns reflect an animal's ecological response to various chemical, physical, or biological stimuli within the environment (Able 1991, Patterson et al. 2008). Such movement responses also reflect the spatial and temporal scales at which species interact with the scale of environmental heterogeneity (patchiness) of the landscape (Bond 1980; Loehle 1990; With and Crist 1995, 1996; Swihart et al. 2008; Fearer et al. 2009; Eccard and Liesenjohann 2008). For example, how resource patchiness is perceived by an organism can be determined by examining shifts in movement behavior associated with being inside versus outside resource patches. Outside resource patches, animals typically use a ranging search behavior, which increases their probability of encountering patches. Once inside a resource patch, however, animals often switch to a pattern of area-restricted search, in which they reduce movement and engage in more concentrated search patterns that help them remain within the patch (Kareiva and Odell 1987, Fauchald and Tveraa 2003). Analysis of animal movement pathways can thus help uncover how an organism perceives the patch structure of a landscape (i.e., spatial heterogeneity; With 1994a, Wiens et al. 1995).

Although the effects of resource patchiness on animal movement have received a good deal of study in ecology, we know far less about how animal foraging activities alter the patchiness of resource landscapes. Especially in terms of how spatial heterogeneity might be generated by foraging within an initially homogeneous resource distribution. Within a homogeneous resource landscape, the interaction between foragers and their resource can give

rise to increasing spatial heterogeneity over time (Utsumi et al. 2009). For example, predators may create areas of high and low prey density as they forage within a landscape (Sih 1984, Fauchald et al. 2000), and herbivores can enhance the heterogeneity of their resource landscape through selective grazing of certain plant species or by preferentially grazing within certain areas of the landscape (Knapp et al. 1999, Senft et al. 1987). In addition, intraspecific interactions—involving either positive (e.g., attraction) or negative (e.g., avoidance or aggression) interactions among individuals—can also influence animal movement and foraging behavior, and thus influence pattern formation within resource landscapes (Franco and Harper 1988, Liao et al 2015). Differences in foraging phenotype may lead to a drastically different patterns of landscape use and feeding behavior (Sokolowki 1980; Niitepold et al. 2009). Comparing a phenotype that has to optimize both the movement within and between patches (exploiter-disperser) to a phenotype that only has to optimize movement within a patch (exclusive-exploiter) gives us the opportunity to understand different strategies and how that might affect fitness. Assuming that the exclusive-exploiter phenotype is more mobile within the patch (Cordeiro et al. 2016), we expect a more random pattern of space use compared to the exploiter-disperser phenotype because that organism has the potential to cover wider area being observed and feeding on different locations.

The relationship between spatial pattern and animal movement is thus reciprocal and dynamic. Elucidating the nature of the relationship between spatial pattern and ecological processes can be difficult to achieve, given that the spatiotemporal scales over which such pattern-process interactions occur can make experimentation and hypothesis testing a challenge if conducted at traditionally defined landscape scales (Turner 1989, 2005). One solution is therefore to develop appropriately scaled experimental model systems using small organisms to

test hypotheses regarding mechanisms and develop insights into the nature of the interaction between pattern and process (Wiens and Milne 1989, Ims et al. 1993, Turner 2005). In that tradition, we therefore developed an experimental model system consisting of a homogeneous resource landscape in which we explored how animal movement behavior alternately gave rise to and responded to spatial heterogeneity (patchiness) over time, and how interactions with the landscape are influenced by the presence of conspecifics of either the same or opposite sex.

Our experimental system consisted of a homogeneous resource landscape (a uniform layer of wheat grain) in which we tracked and analyzed the movement pathways of a seed predator, the lesser grain borer, *Rhyzopertha dominica* (Coleoptera, Bostrichidae), which exploits intact seeds as a food resource, both in nature as well as in human created grain storage systems (Edde 2012). This beetle by feeding on the kernel damages it and can potentially create heterogeneity. Although we predicted that beetles foraging on grain kernels would create localized patchiness within the environment over time, it was less clear how the presence of conspecifics would modify foraging behavior and the development of spatial heterogeneity within the resource landscape. For a species like the lesser grain borer that use pheromones for communication, interactions between conspecifics need not be direct to have an effect on movement behavior. Further, the movement of individuals through an area can leave a chemical signature that alters the future behavior of beetles that move into that area, although perhaps in different ways for males versus females. For example, males may be attracted to females and thus move in ways such that they converge, whereas individuals of the same sex may avoid each other so as to reduce competition and overexploitation of the food resource. Our experiments were thus designed to address: (1) how the movement patterns of this seed predator deviates from random search within an initially homogeneous resource landscape; (2) how beetle

movement patterns change over time in response to increasing heterogeneity in the resource landscape; (3) how the presence of conspecifics (male or female) alters individual movement behavior compared to when beetles are foraging in isolation; and, (4) whether the nature of the relationship between movement behavior and spatial heterogeneity is at all influenced by different phenotypes (wild versus lab-reared strains of beetles).

Material and Methods

Experimental model system

The lesser grain borer is a seed predator and major pest of stored grains (Edde 2012). Although this species can be found exploiting seeds such as acorns in nature (Potter 1935; Wright et al. 1990), it is most often found associated with stored grain in the human-built landscape, where grain is stored in large quantities (e.g., within bags, storage bins, silos, and warehouses) that are patchily distributed across a range of scales on the landscape. Typically, borer insects infest the grain after being placed in storage, rather than in the field prior to harvest, which attests to the strong flight and dispersal abilities of this species. Once a grain store has been colonized, however, it provides a relatively homogeneous resource for *R. dominica*, at least initially.

We used two *R. dominica* strains in this study. The 'wild' strain (predicted to be an exploiter-disperser) was collected in Otwell, Arkansas (July 2012), and had been cultured in the laboratory for ~9 generations before the start of experiments. The 'lab' strain (predicted to be an exclusive-exploiter) was originally collected in eastern Kansas (1972) and has been kept under laboratory conditions for more than 448 generations. Laboratory culture methods consisted of maintaining beetles in 950-ml enclosed jars with 250 g of wheat, which is periodically sieved and a sub-sample of adults placed in fresh wheat. The lab strain has thus been living at relatively

high densities (i.e., more than 300 individuals per culture jar) from which it could not disperse for many generations, giving us the opportunity to compare strains under very different selective pressures. Both strains were reared at $28 \pm 1°C$ and 65 ± 5 % (r.h.) on whole hard red winter wheat as described in Cordeiro et al. (2016), and sex was determined using the methods proposed by Crombie (1941).

For all experiments, we focused on the movement behaviors of adults that had recently emerged from infested wheat kernels. Female grain borers deposit eggs singly or in clusters (up to 30 eggs) in the grain mass. Upon hatching, the first instar larvae tunnel into a seed kernel, where they then complete development before emerging as adults some 4-6 weeks later. Because wheat kernels with *R. dominica* developing inside exhibit no visible sign externally, it was necessary to x-ray the wheat to visualize individuals within the wheat kernels. Infested kernels identified by x-ray were kept individually in glass vials (outer Ø=15mm; inner Ø=13mm; height=45mm) sealed with a cotton ball until the adults had chewed a hole large enough to leave the kernel. Adults tend to remain inside the wheat kernel after this point for a variable number of days before dispersing. During this pre-dispersal time, individual beetles were gently removed from the kernel and marked with a small dot of water-based ink on the pronotum. We used different colors to distinguish individuals during experimental trials. Marked beetles were then returned to the vial, and all re-entered the wheat kernel where they remained until the start of experiments. Because the process of determining sex could injure an adult beetle, we did not assess the sex of individuals until the end of each trial (i.e., experimental treatments were designated post hoc).

We created a homogeneous resource landscape by spreading a monolayer of wheat kernels between two glass plates (40 cm x 20 cm), between which we had inserted plastic strips

(1.5-cm wide and 1.1-cm thick) at the top and bottom so as to create a 5-mm gap to hold the wheat (Fig.1b). The entire assembly was held together using four binder clips placed in pairs along the sides. The resource landscapes each held approximately 250 g of wheat, with a gap of 5 cm between the top of the grain and the top of the glass plates to simulate the surface of a grain patch (e.g., within a storage bin). The dimensions of the resource landscape were sufficient to allow beetles to move freely among the gaps between kernels in both vertical and horizontal directions, much as they do while foraging within a grain mass, while still allowing the position of the beetle and location of feeding damage to be observed through the glass (Cordeiro et al. 2016).

During trials, the resource landscapes were placed vertically inside a box, and the top of the box was then covered with aluminum foil such that only the tops of the plates (~5 cm) were above the foil. This enabled light to reach the top of the grain within the resource landscape, thereby simulating the light gradient that is typically found near the surface in a large grain mass (e.g., a storage bin). Fifteen plates were set up at the same time in the box (a single block), and a given experiment was repeated in two blocks, with each plate representing a single trial. The experiments were conducted at 14L:10D (no dawn or dusk), 60% r.h., and 28°C in an incubator.

Either one infested kernel was placed at the center or two infested kernels were positioned 10 cm apart horizontally along the center of a resource landscape. When two kernels were used, the individual beetles had been marked with different colors to permit the tracking of each individual. During a trial, the sequential locations of each beetle were marked and numbered on the glass plate, once a day for 28 consecutive days. At end of each trial, we photographed the glass plates with the marked beetle positions and then imported the digital images into the image-processing software, ImageJ, which we used to measure the distances and

angles between the sequential locations for each beetle (i.e., movement pattern). From this information, daily displacement and path tortuosity were calculated as described below. In addition to movement patterns, we also characterized the foraging sites (patches) created by *R. dominica*. When feeding on wheat, *R. dominica* create distinct patches, with visible accumulations of flour and frass, which thus add structure to the initially homogeneous resource distribution (Figure 1a). The number and size of these feeding sites were evaluated from the digital images of the plates at the end of the experiment. The size of foraging patches was measured as the area of a circle that completely circumscribed each feeding area.

Is movement random within a homogenous resource landscape?

To test if the pattern emerged from movement and feeding behavior in a homogeneous resource patch is random, we calculated density functions using second-order statistics (i.e., variance of all point-to-point distances) and used Ripley's L-function to test for spatial randomness (Ripley 1988). We evaluated each type of interaction pattern using the point-coordinate data for beetle movements, feeding sites, and the movement and feeding-site combined. Fitted L-function curves were compared to each respective Poisson reference curve, which gives the expectation for a random distribution. If the deviation of the fitted curve from the reference curve was positive, above the upper limit of the confidence interval, the distribution was clumped. If the deviation from expected was negative, below the confidence interval, the distribution was overdispersed. If the fitted curve fell within the bounds of the confidence interval for any given *t*, the null hypothesis of complete spatial randomness cannot be rejected (Haase 1995). Due to the large number of L function diagrams generated (one per replication), data are presented as frequency diagrams that represent the probability of finding each type of pattern (clumped, random or overdispersed) at each scale as a function of sex.

The L-functions were generated for each microlandscape by using 'spanc' package (Rowlingson and Diggle 2013) within the statistical software R (R Development Core Team 2013). Ripley's K or L functions have been primarily used to describe the spatial pattern of plant species (Pyllay and Ward 2012, Fibich et al. 2016), but this approach can also be suitable for study of animal movement and interaction with the environment since these interactions can also be exploited at different spatial scales.

Do changes in landscape heterogeneity due to foraging behavior affect subsequent movement pattern?

Cordeiro et al. (2016) reported that the *R. dominica* often was observed near previous feeding sites and occasionally returned to the kernel from which they had emerged. Recognition of sites where they have fed previously may thus influence the movement patterns of *R. dominica*. To specifically test if insects show a preference for previous feeding sites, two experiment tests using a two-choice design were conducted. For these tests, we collected 5 g of wheat from within feeding sites and 5 g of wheat from non-feeding sites within monolayers used by each individual in the previous experiment. The first test evaluated preference by beetles toward wheat from their own feeding sites compared to non-feeding site wheat. The second test evaluated beetle preference for wheat from feeding sites created by another individual compared to their own feeding sites. For both tests, selected wheat was placed in 35 x10 mm Petri dishes with six equidistant openings (Ø=3 mm; 1 mm above the ground) drilled on the sides to allow beetles to enter and exit dishes. The dishes containing wheat for the choice tests were placed on opposite sides of a 9 x 9 cm^2 square Petri dish. Discs of filter paper were fixed at the bottom of the square Petri dish bottom using water-based white (synthetic) glue resin, and the inner walls of the square dish were coated with Teflon PTFE (DuPont, Wilmington, DE, USA) to prevent

beetles from climbing up the sides. The choice tests were prepared and conducted immediately after the end of the microlandscape experiments. To start the tests, the individual beetles were placed at one of the empty corners of the square Petri dish. The choice tests were run for 24-h in complete darkness under controlled temperature (30±1°C) and relative humidity (65±0%). Beetle choice was assigned based on which dish beetle was in at the end of the evaluation. Data was analyzed using a binomial test procedure to evaluate if the frequency of choice was non-random.

Does the presence of conspecifics alter individual movement behavior?

To evaluate association in movement between pairs of beetles, we estimated the multitype K function, which counts the expected number of points of the second insect within a given distance of the subset points of the first insect (Harkness and Isham 1983). The multitype K function helped us to understand the pattern of movement association between the two beetles in the microlandscape (clumped, random, overdispersed).

To test if there are differences in the way isolated males or females exploit their environment and if the presence of another beetle will affect movement patterns, we evaluated two movement parameters: daily displacement and tortuosity. Daily displacement (cm), the net distance traveled in a 24-hr period, was obtained for each individual in treatments with male alone, female alone, and in pairs of the same or opposite sex. Tortuosity is a measure of movement pathway complexity (With 1994a,b), with the fractal dimension (D) ranging between 1 (path resembles a straight-line) and 2 (path pattern covers an entire plane) (With 1994a, Nams and Bourgeois 2004). Tortuosity can sometimes change with spatial scale of measurement, and is therefore considered to be a scale-dependent parameter. When D values are the same across different scales of measurement, the pattern exhibits similar structure across a range of scales; that is, it is fractal (Mandelbrot 1967, Nams and Bourgeois 2004). To compare tortuosity of

movement using the same spatial scale, we estimated *D* for both males and females when

isolated, and when combined in pairs of the same or different sex, using the VFractal software

(Nams 1996). Movement was so limited in some males (n=6) that we were unable to calculate

D, and thus these individuals were not included in the analysis (Caldwell and Nams 2006).

Data were analyzed by ANOVA using type III sums of squares to measure the effect

comparing the six levels of the independent variable (single male, single female, male x male,

female x female, male x female, and female x male) on the response variable daily displacement

or tortuosity followed by post-hoc comparisons of significant effects using Tukey's HSD test.

The analyses were performed using SAS version 9 software (SAS Institute, Cary, NC). All data

are presented as mean ± SEM.

Is the relationship between movement behavior and spatial heterogeneity

influenced by different phenotypes?

Similar to what was done to test spatial randomness in the wild strain, we used Ripley's

L-function also to test spatial pattern over different scale range of movement, feeding sites, and

feeding site and movement coordinates of both females and males from lab strain (Ripley 1988).

Results

Is movement random within a homogenous resource landscape?

The spatial distribution of both male and female beetles and their feeding sites were

dependent on the spatial scale over which they were evaluated (Fig. 2): distribution was clumped

at short range (<10cm), random at intermediate range (10-20 cm), and overdispersed at longer

range (>20 cm). Interestingly, males and females showed similar spatial pattern, but differed in

the range of each pattern. Males had a predominantly clumped phase that extend for a longer

distance than females, that had a broader random phase revealing a greater tendency of males to be associated with the clumped pattern and females with the random pattern.

Do changes in landscape heterogeneity due to foraging behavior affect subsequent movement pattern?

Regardless of sex, beetles showed a strong preference for wheat collected from feeding sites rather than wheat from non-feeding sites (86.4 % vs. 13.6%, respectively, z=3.4, P<0.001, n=22). This preference was not specific to their own feeding sites, as there was no significant preference for wheat obtained from their own feeding site versus wheat from another individual's feeding site (45% vs. 55%, respectively; z=-0.89, P=0.37, n=20). Beetles preferred feeding-site patches regardless of whether it was produced by a beetle of the same or opposite sex ($F_{3,16}$=0.44, P=0.73).

Does the presence of conspecifics alter individual movement behavior?

The spatial distribution of males and females was affected by the presence of other individuals of either the same or opposite sex (Fig. 3). In relation to another female, females exhibited a random distribution across all scales (Fig. 3). The behavior of one female thus does not seem to induce any specific pattern to the movement or feeding sites of another female. In relation to another male, males also exhibited a random distribution, except at distances less than 10 cm, in which nearly 20% of males exhibited a clumped distribution, suggesting that males were interacting with other at close range. In relation to the opposite sex, however, individuals exhibited a more clumped than random distribution at most distances, with the greatest degree of clumping occurring at an intermediate scale (10-15 cm).

There was a significant effect of treatment on daily displacement ($F_{5,68}$=6.55, P<0.001; Fig.4). The presence of another female significantly increased female daily displacement and this

combination had the highest calculated displacement (mean=9.7±1.1 cm/day, n=8, $P<0.001$; Fig. 4). Females by themselves (mean=7.2±0.6 cm/day, n=18) were about twice as mobile as solitary males (mean=3.7±0.9 cm/day, n=14, $P<0.001$) or males paired with another male (mean=3.0±1.1 cm/day, n=5, P<0.011). Males alone or paired with another male did not differ in net displacement ($P=0.681$). Males in the presence of a female (mean=4.6±1.2 cm/day, n=9) or females in the presence of a male (mean=4.6±0.9 cm/day, n=9) were intermediate in displacement and did not differ statistically from each other ($P=0.984$) (Fig. 4).

Movement path complexity was different between males and females ($F_{5,68}=3.51$, $P<0.007$; Fig. 5). Females had a more tortuous path than males and consequently higher values for fractal D. Path tortuosity for females (mean=1.4±0.0, n=18) was not significantly affected by the presence of another female (mean=1.4±0.0, n=8, P=0.50) or by a male (mean=1.5±0.1, n=9, P=0.43). Males had a more linear and less complex path than females (mean=1.2±0.0, n=14). The presence of a female significantly increased fractal D values of males (mean=1.3±0.1, n=9), indicating that males detected the presence of females and changed their movement paths so they were more similar to females (Fig. 5). Males did not change their movement behavior in response to the presence of another male (mean=1.2±0.1, n=5, P=0.78). We were not able to calculate D for all replicates due to males' low movement rate.

When beetles were in monolayers with another individual of the same sex, individuals maintained an average distance of 12.3 ±1.1 cm in the case of two females (n=9) or 11.1±1.0 cm (n=9) for two males; these distances were not significantly different (n=19, P=0.53). However, individuals were 2x closer when paired with an individuals of the opposite sex (mean=5.4±1.0 cm, n=9, $F_{2,25}=11.45$, P<0.001).

Is the relationship between movement behavior and spatial heterogeneity influenced by different phenotypes?

The resultant spatial pattern left by the two distinct phenotypes was different at the intermediate and large scale (Fig. 6). Lab strain males and females exhibited a predominantly clumped distribution up to a distance of 20 cm. At longer distances, lab strain sexes differed in distribution with a predominantly random distribution for females and a combination of random and overdispersed pattern for males (Fig. 6).

Discussion

In our study, a clear link can be established between an animal's movement patterns and the formation of spatial heterogeneity within a homogeneous resource landscape. Our findings on the movement and feeding behaviors of a seed predator, the lesser grain borer (*R. dominica*), indicate the existence of at least two, non-mutually exclusive mechanisms that contribute to pattern formation. First, individuals interact with their habitat by feeding within the resource landscape, creating variation (patchiness) that then affects subsequent beetle movement and space use. In this case, feeding sites (created either by themselves or another beetle) were more attractive to foraging individuals than un-exploited sites, and therefore created a positive feedback mechanism by which spatial pattern (patchiness) emerged within the initially homogeneous resource landscape. Second, individuals may alter their movement behavior in the presence of other beetles. Conspecifics can have either a negative effect on each other, as when females increase their displacement rate in the presence of another female, or a positive effect, as when males seem to follow females and become more "female-like" in their movements (i.e., decreasing distance between each other, and increasing displacement and *D* values). The

presence of other individual of the same or opposite sex can further influence distribution patterns (of individuals or their feeding sites) and the scales at which patchiness emerges.

Although much research has examined how species respond individually to environmental heterogeneity (e.g., analysis of individual movement pathways in different landscape contexts; Crist et al. 1992; With 1994a,b; With & Crist 1995; Etzenhouser et al. 1998; Frair et al. 2005) or how the foraging activities of species can enhance heterogeneity within landscapes (e.g., grazers; Addler et al. 2001), few studies have explored how intra-specific variation and possible interactions can reinforce the reciprocal effects of pattern on process (e.g., With (1994a) studied different developmental stages). Our study demonstrates that the presence of a conspecific can have a significant impact on movement parameters, affecting either the rate or complexity of movement pathways, which then manifest as fundamentally different patterns of space use and heterogeneity in the resulting resource landscape.

Isolated male and female beetles exhibited fundamentally different movement behaviors, demonstrating an intrinsic source of variation in behavior that can potentially modulate the way they interact with their environment and help explain observed spatial patterns. This sort of variation is often considered to be "statistical noise," and thus its ecological and evolutionary implications are generally underestimated and under-represented in the literature (Sih et al. 2004). After emerging within a homogeneous resource landscape, isolated females tended to travel farther on a daily basis and to exhibit a more complex movement pathway (i.e., higher D values) than isolated males. More complex movement pathways are likely an indicator of more intensive foraging behavior and microhabitat selection (With 1994b, Caldwell and Nams 2006, Nams 2006a,b).

Male *R. dominica* are known to produce an aggregation pheromone, to which both females and males respond (Khorramshahi and Burkholder 1981, Williams et al. 1981). Therefore, intersexual differences in movement may come about primarily because of different mate-finding strategies in males versus females (Gehrt and Fritzell 1997). Males may move less because they are spending most of the time feeding, which has a direct correlation with pheromone production (Bashir et al. 2006), and because calling from a fixed location increases their chances of being found by a female. This behavior seems to be conditioned to the absence of the female in close range or previous contact with the female, since all the metrics change when the opposite sex is present. Conversely, females exhibited more complex search patterns, presumably because they are attempting to locate the less-mobile males. Thus, our study of individual movement patterns of single beetles can be interpreted as the "default" behavior during the initial colonization and exploration of a resource landscape (Lima and Zollner 1996).

Despite these differences in movement behavior, male and female beetles exhibited similar space-use patterns specially in short range with males having a wider range of distances over which they exhibit a clumped distribution and a narrower range in which they have a random pattern. An animal can respond differently to its environment at different spatial scales, signifying different domains of scales, in which case the structure of its movement path is predicted to change from one scale to another (Wiens et al. 1993). One way to detect domains of scale is to test for deviations from randomness across multiple scales (Haase 1995, Fortin et al 2013). The spatial distribution of beetle movement locations and feeding sites were scale-dependent, and an analysis of the resulting spatial pattern enabled us to determine the distances over which different cues might be able to influence movement behavior. For example, our study indicates that beetles respond to cues from feeding sites at distances <10 cm, and move randomly

beyond that distance. Kernels damaged by feeding offer less resistance to additional feeding, and the damaged kernels and accumulated frass release volatile chemical cues that make them more attractive to beetles (Dowdy et al. 1993, Edde and Phillips 2006).

Although, the majority of beetles exhibited random movement at an intermediate scale (10-20 cm), most beetles showed an overdispersed pattern at distances >20 cm. It is difficult to explain why this shift in the pattern of space use occurs, but one possibility is that there is a concomitant shift in the way beetles move, from a random to a more directed and linear pattern deduced from the cluster pattern of feeding sites distributed in a random or overdispersed manner across the landscape. Although we did not directly test this hypothesis, the assumption is that beetles may alternate between randomly searching within a given area (patch) that are then interspersed with longer-range movements. The overdispersed pattern of space use was stronger in males and in the wild strain, both of which tended to be less mobile overall than either females or the lab-reared strain (Cordeiro et al 2016).

The paired-beetle experiments showed that interactions between individuals might also play a role in the way individuals move through these resource landscapes. In the presence of another female, females significantly increased their rate of displacement. It is not clear what cues they used to detect the presence of other females, since only males have been reported to produce pheromone (Williams et al. 1981). It is possible that an unidentified female produced pheromone is present, but in the absence of this it is also possible that they detect tactile cues associated with activity by another individual. Given that females are more active than males and get even more active in presence of another female, those indirect clues might explain the differences in response by females to other females. By contrast, the presence of another male did not significantly affect the overall movement of males, which can give us extra support to

idea that aggregation pheromone elicits a limited functional response in other males when those males are already within a resource patch (Cordeiro et. al 2016b, *in preparation*).

In our experiment, the presence of a male did not affect movement parameters of another male (i.e. daily displacement or D), however, the pattern of one male in relation to the other male exhibits clumping at short scale (distances < 10 cm). Males produce aggregation pheromone, which attracts both sexes similarly outside of the grain (Williams et al. 1981, Dowdy et al. 1993), but our results presented here and in Cordeiro et al. (2016b) suggest that while males show indications of detecting pheromone over very short distances, it has a very limited influence on their movement behavior. On the other hand, the space-use pattern of two females in relation to each other was random at all scales, suggesting that females do not interact with each other in these resource landscapes. The fact that an analysis of their movement patterns revealed that females increase their daily displacement and tortuosity of movement in the presence of another female, however, suggests that they are exerting some influence on each other, just not in a way that changes their spatial association.

When males were paired with a female in these resource landscapes, the complexity of the male's movement pathways show a tendency to increase relative to isolated males and when paired with another male. The resulting space-use pattern of the paired male and female changed with scale, but was largely clumped. Since the space-use pattern of males changed in the presence of females relative to their space use when isolated, this suggests that they detected the presence of the female, and furthermore, converged in their movement and space use on females. For male *R. dominica*, this may entail switching from a stationary-call strategy to an active-search strategy when females are detected. Animals will tend to increase search effort after detecting a resource (i.e. mating opportunities), as an individual is more likely to find additional

resource in that same vicinity, but should decrease search effort in that area once it reaches a point of diminishing returns (Pyke 1978, Pyke 1984, Benhamou 1992, Fielden et al. 1990).

Due to different selection pressures on the lab versus wild strains, we expected that this would contribute to differences in the way beetles exploited their resource landscape. Under conditions of high density over many generations, such as in the lab-reared strain, a response to chemical cues may be selected against (Sgrò and Partridge 2000). In addition, the culturing methods used in the laboratory to maintain these colonies, which involves randomly obtaining (sieving) individuals with which to start the next generation, tend to select against dispersal and favor increased reproduction. In accordance to our expectations, the lab strain had a predominantly clumped pattern within the <20-cm range and was random otherwise, whereas the wild strain had a clumped pattern of space use at distances <10 cm and a random pattern within a narrower range (10-20 cm) compared to the lab strain, forming an interesting contrast between the two strains. A marked difference between the two strains is the higher mobility of the lab strain that is associated with a better ability to explore the environment can potentially explain the differences in spatial pattern formation. Lab strain would probably perform poorly in movement between patches were the insect has to interact with matrix of a different material (Cordeiro, *non-published data*). The ability to engage in a wider foraging range is likely associated with a lack of tradeoff between movement within patch and between patches for the lab strain (i.e., ability to explore new environments, to flight or to detect chemical clues in low concentration in nature), which may be related to the inadvertent selection over generation for foraging within a homogeneous resource (Hoffmann et al 2001, Linnen et al. 2001).

Conclusions

Clearly, non-random foraging behavior can give rise to spatial heterogeneity within an initially homogeneous resource landscape. However, individuals rarely forage or live in isolation. More interestingly, then, is the extent to which the presence of other conspecifics—and whether these conspecifics are of the same or opposite sex—alters movement behavior, space use, and thus the formation of resource patchiness. In the case of the lesser grain borer, a seed predator and major grain pest, we have demonstrated that besides intersexual differences in movement and space use, differences in the social environment can also contribute to differences in resource utilization that affect the resulting patchiness of the resource landscape. Although these differences can be explained in terms of different motivations (resource exploitation vs. reproduction), they nevertheless underscore the wide range of individual behaviors that may be encountered within a population, which can frustrate efforts to translate from individual movement behaviors to predict the spatial distribution of populations (e.g., With & Crist 1995, 1996). In addition, different populations may experience different selective pressures on dispersal or resource utilization, which can likewise affect interactions between individuals and their resource landscape.

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Figures and Tables

Figure 4.1 (a) Example of a progression of feeding site formation over time after the lesser grain borer, *Rhyzopertha dominica*, adult emergence from kernel in a quasi-homogeneous medium (mono-layer of wheat). (b) Examples of single male and single female movement and feeding patterns in quasi-homogenous medium. Gray lines represent the insect daily position over the course of 28 days. Full black circles indicate the position of the infested kernel that beetle emerged from and the open circles represent feeding sites created by the insect. Different diameters represent different feeding site sizes produced by the beetle.
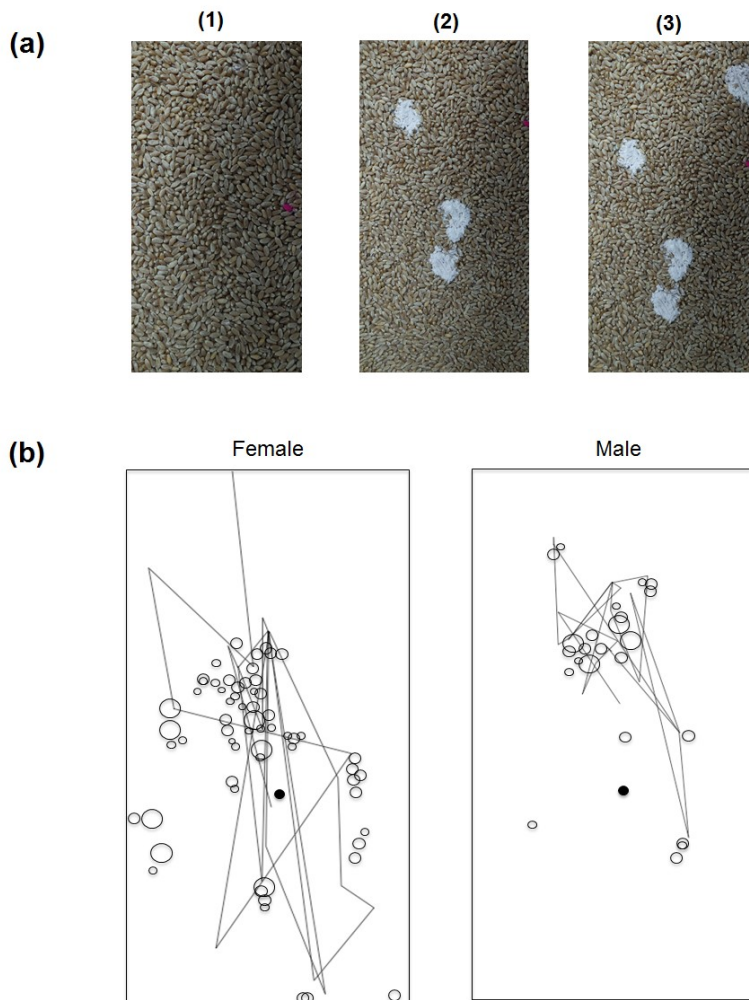
Figure 4.2 Frequency of the three classes (random, clumped, overdispersed) of pattern observed in 32 K(*t*) plots for the wild strain *R. dominica* using movement, feeding sites, or movement and feeding site coordinates combined. Classes were determined of each individual K(t) plot at the same distance interval comparing the fit curve with Poison theoretical curve envelope.
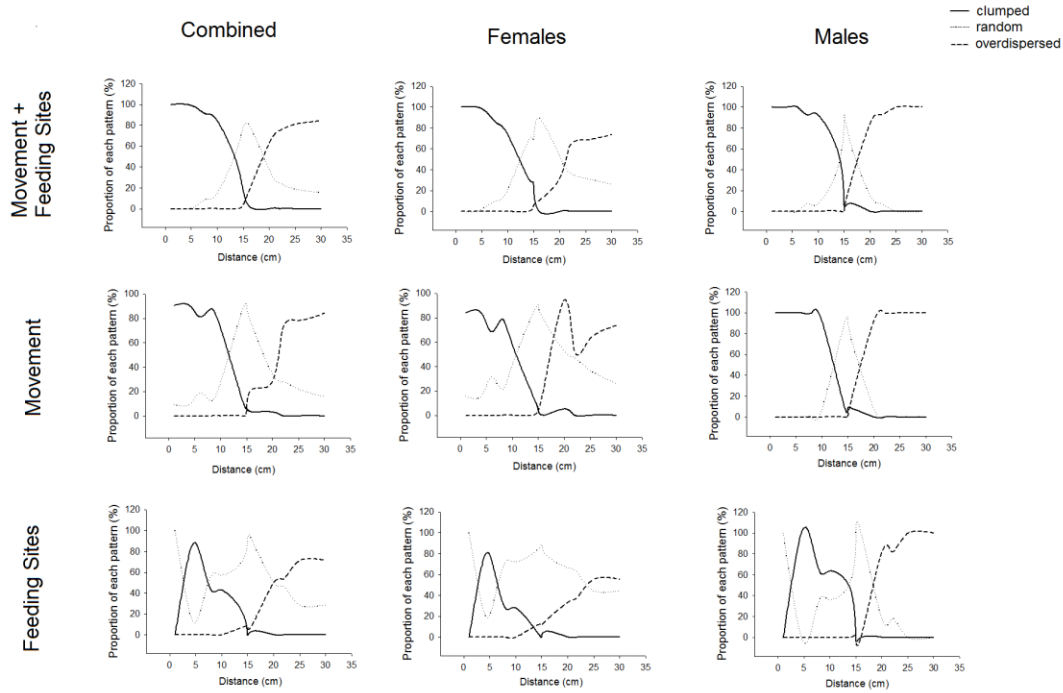
Figure 4.3 Frequency of the three classes (random, clumped, overdispersed) of pattern observed in 22 K(t) plots for the lesser grain borer pairs using movement position of pair individuals of the same or different sex. The point pattern set of was individual was compared to the point pattern of the other better. Classes were determined for each individual K(t) plot at the same distance interval comparing the fit curve with Poison theoretical curve envelope.
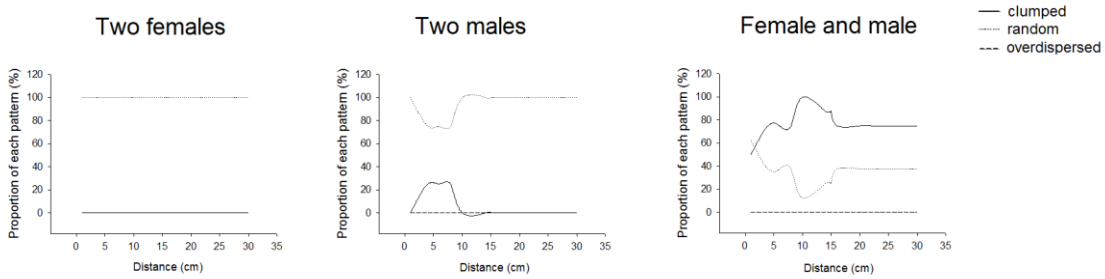
Figure 4.4 Daily displacement (mean±SE) of R. dominica as a function of the sex and presence of conspecific (White = single insect, gray = pairs of different sex, black = pair of the same sex) ($F_{5,68}$=6.55, P<0.001). Tukey's Studentized Range (HSD) test was used for post hoc comparison, different letters represent significance at 0.05 level.
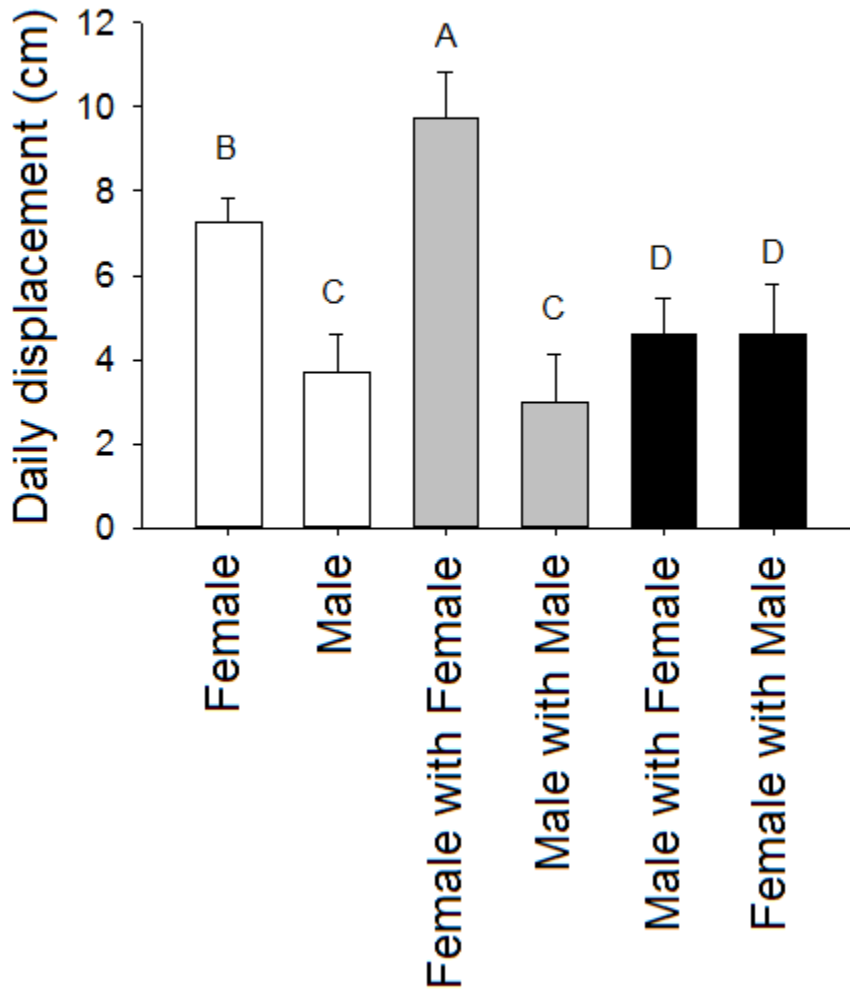
Figure 4.5 Fractal dimension (mean±SE) of lesser grain borer, *Rhyzopertha dominica*, movement as a function of sex and presence of conspecific (White = single insects, gray = pairs of different sex, black = pair of the same sex) ($F_{5,68}$=3.51, *P*<0.007). Tukey's Studentized Range (HSD) test was used for *post hoc* comparison, different letters represent significance at 0.05 of probability.
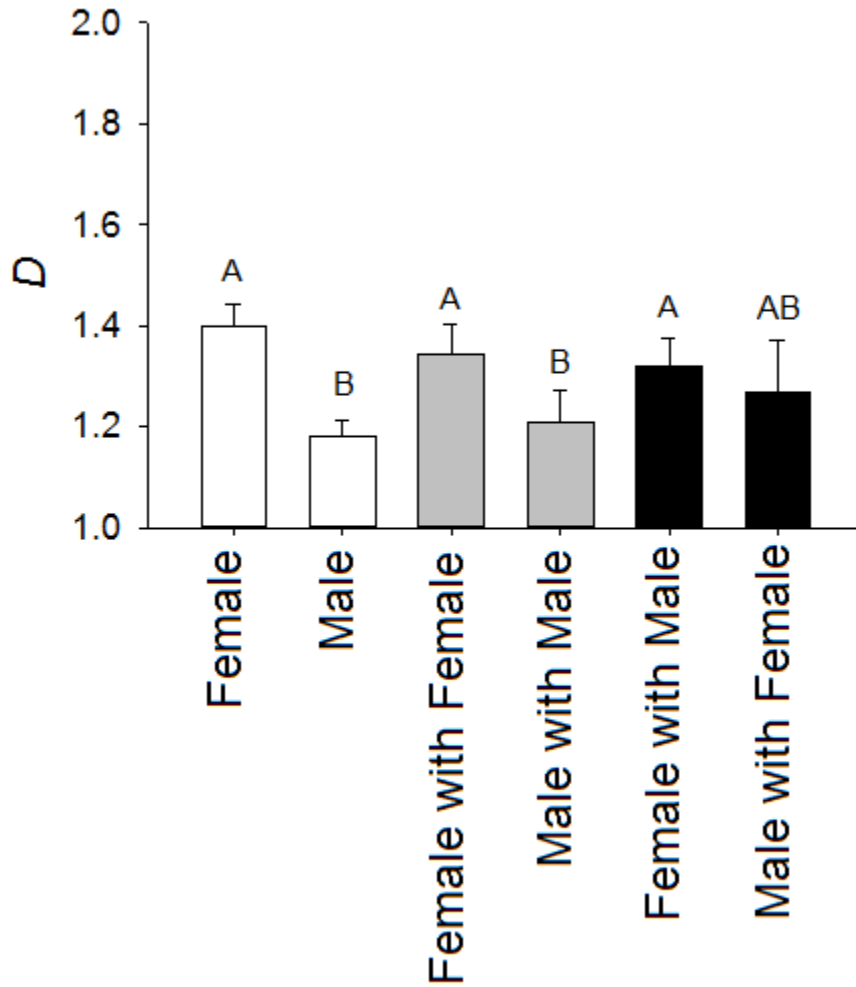
Figure 4.6 Frequency of the three classes (random, clumped, overdispersed) of pattern observed in 20 K(t) plots for the lab strain lesser grain borer using movement, feeding sites, or movement and feeding site coordinates combined. Classes were determined for each individual K(t) plot at the same distance interval comparing the fit curve with Poison theoretical curve envelope.
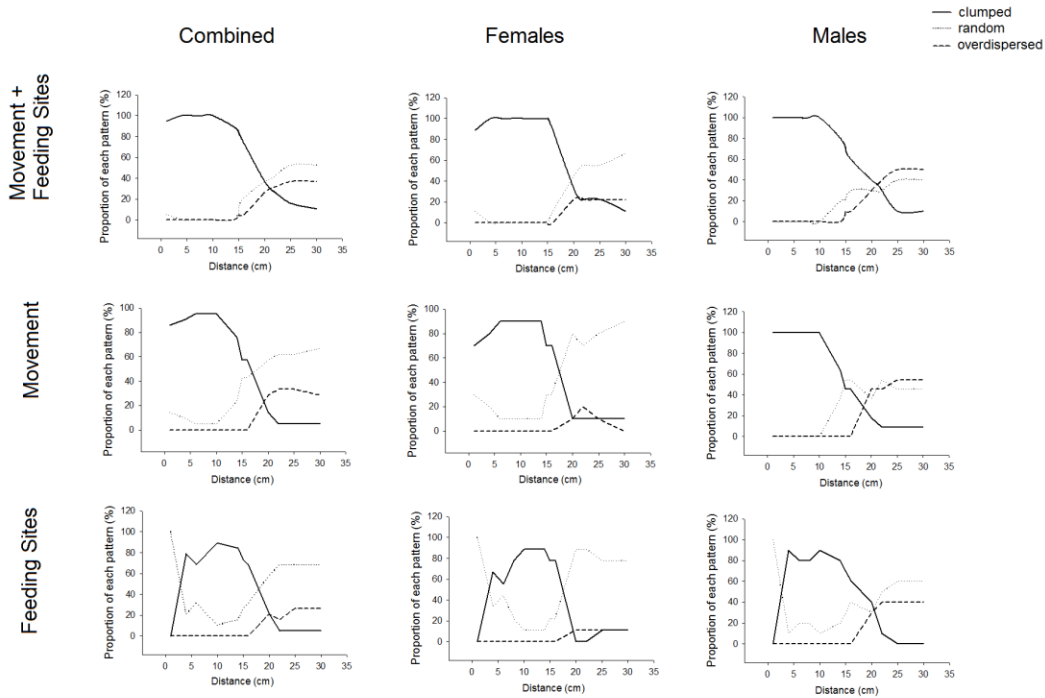
Figure 4.7 Ecological model demonstrating different spatial pattern between exploiter-disperse strategy (wild strain) and exclusive exploiter strain (lab strain) in movement within the resource patch derivate for *R. dominica* movement and spatial pattern.
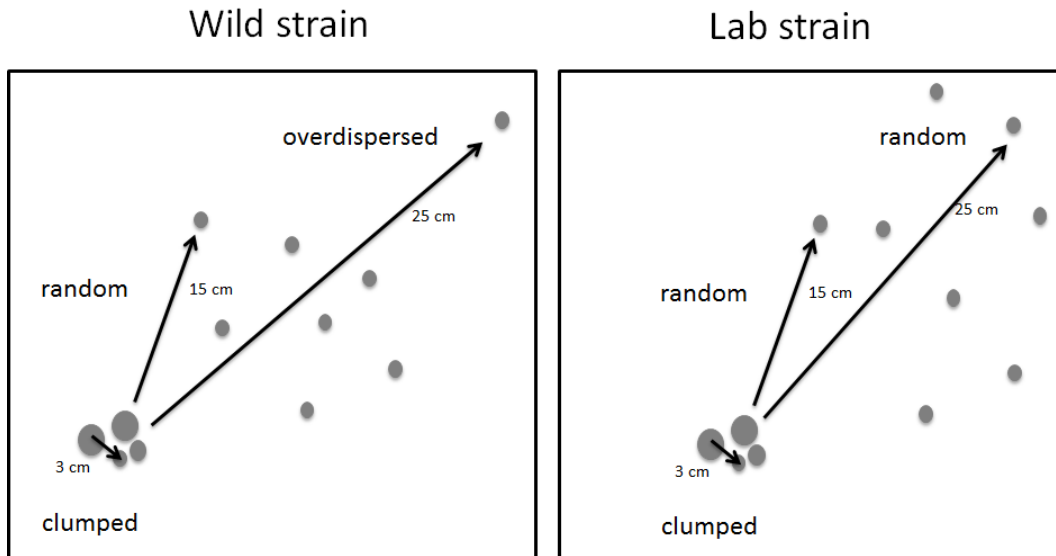
Chapter 5 - Source-sink population dynamics and dispersal facilitation by trade routes: impact on population structure of a grain pest

Abstract

For insect pests of wheat and other grains, population structure is likely impacted not only by geographic distance and ecoregions, but also by patterns of grain production and distribution (i.e., different regions of the United States are potential sources and sinks depending on levels movement of grain into and out of region). We used 209 *Rhyzopertha dominica* beetles collected at 11 different sites and sequenced using double digest RADseq to generate 5379 SNP markers to assess the genetic diversity and genetic structure of putative populations. Genetic data indicated that nucleotide diversity values were similar among populations, but tended to be lesser in small wheat production areas and greater in large wheat production areas. Ecoregion was more important in explaining structure of *R. dominica* populations (F_{SC}=0.023, p<0.001) than crop type (i.e., rice or wheat regions) (F_{CT}=0.003; p> 0.05) according to AMOVA hierarchical analysis. Although isolation by distance (IBD) was significant according to the Mantel test (r=0.68; p= 0.003), a substantial degree of admixture between populations was found. Model selection approach elected the volume of grain received, the wheat acreage, and the average geographical distance to other locations as the most important variables to explain the degree of population differentiation and diversity found in *R. dominica* populations in the United States. We conclude that *R. dominica* populations experience isolation by distance, and have genetic structure explained by the ecoregions rather than crop regions which indicates movement between wheat and rice production regions. However, the degree of genetic proximity and the

admixture is largely affected by grain movement by railroads and the acreage of wheat grain in the surrounding area. The source-sink dynamic has been suggested as a suitable model to explain the dynamic of grain movement in the U.S., and it seems to have a significant impact on insect population associated with grain commodities as well.

Introduction

The increasing demand for commodities and the differences in crop aptitude to a specific geographic region make us more dependent upon a complex transport network that connects food storage facilities across globe. However, storage sites represent a potential source of resource for insects that can become an obstacle to food security when they are able to colonize and growth within those storage facilities (Godfray *et al.* 2010). Insects take advantage not just of the artificial resource patch, but also use the complex network transport system to disperse to remote locations. The adaptations required by natural populations to become a primary pest to a certain crop or storage site, the relative importance of the transport network over flight dispersal on population structure, and if different crops can be a factor structuring polyphagous insect populations are still largely unknown (Via 1990; Estoup & Guillemaud 2010; Nopsa *et al.* 2015).

Rhyzopertha dominica, the lesser grain borer, is one of the major insect pests of stored grain throughout the world (Potter 1935). Grain such as wheat and rice typically becomes colonized by *R. dominica* after it is harvested and stored, with sources coming from either residual population on site or movement of beetles into the storage by their own flight dispersal or transportation in grain and grain handling equipment. This species can also be found in natural areas feeding on fruit seeds, shrub, dry-wood, and timber (Potter 1935; Wright *et al.* 1990; Jia *et al.* 2008); however, the role of natural habitats on dynamics in grain storage is not well understood (Mahroof & Phillips 2007; Ching'Oma 2006). *R. dominica* is a strong flier and cross-

infestation among storage sites or from natural areas to storage sites is possible (Edde 2012; Ching'Oma 2006), but little is known about their long range dispersal ability (Sinclair & Haddrell 1985; Dowdy & McGaughey 1994; Toews *et al.* 2006).

R. dominica is found throughout the United States and can be found in all major wheat and rice areas, although it is a less abundant and a less serious pest in the northern parts of the U.S. and Canada (Edde 2012). Due to its broad geographical distribution, it is present in many of the ecoregions within the United States, including diverse habitats such as prairies, plains, mountains, valley, highlands, hills, and lowlands (EPA 1997). The ecoregions could potentially shape local populations of *R. dominica* according to the adaptations requirement to a certain region, but the environment within grain storage is likely very homogenous across these ecoregions which could lead to more homogeneity in populations. In addition, due to the extensive movement of grain, especially wheat, by roads, railroads, and fluvial transportation, ecoregions may be intensively interconnected.

Insects associated with stored grain can have their long range dispersal facilitated by transportation from farm storage, through the elevator system, to end users such as mills and other processing facilities, thus the transport network may have a significant impact on population structure (Drury *et al.* 2009; Semeao *et al.* 2012; Nopsa *et al.* 2015). A source-sink model has been proposed to explain the grain movement dynamic between large producers and consumers (Nopsa *et al.* 2015). Source-sink dynamic predictions are largely based on the differences in patch quality (i.e. higher in the source and poorer in the sink population) (Dias 1996). Individuals in a source population have a greater rate of population growth due to more opportunities to find suitable habitat where it can feed and reproduce, and the opposite is expected to sink populations (Diffendorfer 1998). Over a long period of time, the source-sink

model predicts that the source habitat is a net exporter of individuals and the sink is a net importer, with the assumption that dispersal is constrained so that individuals cannot be at any possible location (Holt 1997; Dias 1996; Diffendorfer 1998). However, not much is known about how grain production and movement dynamics actually affect local adaptation, population structure, allelic diversity, insect migration, or the relationships between grain producing regions for any stored grain pest insects, including *R. dominica*.

A limited number of studies have been conducted to evaluate stored grain pest insect population structure and these studies have used a range of different markers including mtDNA, a small number of microsatellites, or nuclear gene sequences (Semeao *et al.* 2012; Thagaraj *et al.* 2016; Coelho-Bortolo *et al.* 2016). These studies have typically had a limited number of markers and have found non-significant correlation between genetic distance and geographic distance or been able to identify population structure. The reason for that is attributed to the large amount of human aided dispersal and the limited number of markers to detect pattern given a high background level of movement. Next-generation sequencing (NGS) genotyping methods (Davey *et al.* 2011; Li *et al.* 2012) by generating thousands of markers using reduce genome representation has been successfully used to answer questions regarding population genomics and association mapping (Narum *et al.* 2013). The development of genomic resources such as large SNP data set can be a strong tool helping elucidate highly connected and presumably weakly structured populations (Hess *et al.* 2013; Willet *et al.* 2014). The scarce genetic information available for *R. dominica* makes reduced representation libraries combined with high-density SNP genotyping an attractive method to be used to study complex ecological problems (Baird *et al* 2008; Peterson 2015).

Our primary objective in this study was to estimate the relative importance of natural ecoregions, crop production areas, geographical distance, and human-aided movement through grain transportation networks on *R. dominica* population structure within the United States. We collected beetles from different wheat and rice production regions within the same season and conducted a hierarchical approach to test for possible structure causing factors and model selection (Johnson & Omland 2004; Wagenmakers & Farrell 2004) was used to access the relevance of different factors. Understanding population structure for *R. dominica* will provide insights into potential for local processes of adaptation and broader patterns of movement that will impact management programs and the potential for spread of resistance genes.

Materials and Methods

Sampling

We used *R. dominica* beetles collected at 11 different locations within wheat and rice production areas and within 10 different ecoregions according to (EPA 1997) within the United States (Fig. 5.1, Table 5.1). Sampling was done between July and November 2013, which is the period when most flight activity is reported for this species (Edde *et al.* 2006; Toews *et al.* 2006). Six pheromone-baited delta traps (Scentry Biologicals Inc., Billing, MT) were deployed at each location targeting flying insects in the field near storage sites. The insect traps consisted of a cardboard sheet folded in a triangle shape with inner surfaces coated with sticky glue and containing a rubber impregnated with pheromone lure (Trece Inc., Adair OK) inside the trap. The traps were placed at least 10 m apart from each other and close to grain storage sites such as grain bins, grain elevators, and warehouses where possible. The pheromone lure within the traps can attract both male and female adults flying outdoors (Leos-Martinez *et al.* 1986; Toews *et al.* 2006). The traps were placed in the field for a week before being collected and for most locations

shipped back to our lab where beetles were processed. For processing, the beetles were first carefully removed from the sticky glue inside the trap with forceps and transferred to a 15 ml tube containing 95% EtOH. A histological cleaning agent (Histo-Clear™ II, National Diagnostics; Atlanta, GA) was added to the tube and tube vortexed to remove residual glue from the beetles. The beetles were then transferred to a 1.5 ml tube with 95% EtOH and stored at -80°C until DNA extraction. The number of beetles collected at each location varied due to both the number captured in traps and the integrity of the body after processing the sample. Due to the variation in capture time during the week deployed and environmental conditions dehydration occurred in some individuals which can decrease DNA extraction yield. Beetles that did not remain intact after processing were discarded.

DNA extraction and library preparation

Genomic DNA was extracted from individual *R. dominica* using the DNease kit (Qiagen) following manufacture's recommended protocol. We eluted the DNA using distilled water and spun under vacuum (Speed Vac® SC110, Savant) to 50 μl of concentrate DNA from each sample. RAD-sequencing libraries were prepared following Saintenac *et al.* (2013). Two libraries containing 80 individuals and one containing 96 individuals were barcoded with unique nucleotide sequences were used. Complexity-reduced genomic libraries were prepared using the combination of two restriction endonucleases (RE), *PstI* (CTGCAG) and *MseI* (AATT), to create the genomic DNA fragments. The size distribution of DNA fragments in the genomic libraries and the presence of contaminating adaptors peaks were tested on Bioanalyzer and real-time PCR was used to quantify libraries. After size selection targeting ~300bp DNA fragment length, three libraries (pool of 80, 80, and 96 individuals) were diluted to 10 nM concentrations and

sequenced on three lanes of Illumina HiSeq2000 flow-cell (100bp single-end read run) at the University of Kansas Genome Sequencing Core facility.

Genotyping

Rhyzopertha dominica libraries were demultiplexed, separating individual beetles using the process_rad-tags script in STACKS v.1.35 (Catchen *et al.* 2013). The SNPs were identified on the 100 bp barcoded reads then filtered for overall quality (Ilut *et al.* 2014). A maximum two nucleotide mismatches (M=2) and minimum stack depth of three (m=3) among reads with potentially variable sequences was the parameter used for the formation of RAD loci (Ilut *et al.* 2014; Benestan *et al.* 2015). In population module of STACKS the following filtering steps were taken. We used RAD tags with a minimum stacks depth (m) of 10. Filtering was set to retain SNPs genotyped with at least 85% of the individuals and ~81% of the sampling locations (9 locations) and to remove markers showing heterozygosity greater than 0.5 within samples to avoid potential homologs (Hohenlohe *et al.* 2011). The minor allele frequency (MAF) filtered out alleles with frequency less than 5% (MAF< 0.05) (Roesti *et al.* 2012). Details of the number of SNPs kept after filtering step were stored in VCF, genepop, and structure files. Output files were converted when necessary into other file formats using PGDSPIDER 2.0 (Lischer & Excoffier 2012).

Nucleotide diversity, population history, and migration inferences

Standard diversity indices for each location using SNP set consisting of variant and invariant sites were calculated using the population module in STACKS and genetic diversity analysis in GENODIVE. The effective number of alleles, the observed (H_o) and expected heterozygosity (H_e), heterozygosity within population (H_s), total heterozygosity (H_t), inbreeding coefficient (G_{IS}), private alleles, and nucleotide diversity (π) across the genome were estimated

for each sampled location (Nei 1987). Under a standard neutral model, an ideal diploid population has nucleotide diversity (π) equal to $4N_e\mu$, where N_e is the effective population size and μ is the mutation rate per generation (8.08×10^{-8}). Because there is no estimate of nuclear mutation rate available for *R. dominica* we used approach of O'Loughlin *et al.* (2014). We used the estimation that *R. dominica* has about 7.35 generations per year across our sample locations and a mutation rate of 1.1×10^{-8} per year, estimated from divergence of *Drosophila* linages (Tamura et al. 2004). The estimated effective population size, from $\theta=4N_\theta\mu$, and a measure of migration between locations, (N_em) based on F_{ST} (from $N_em = (1- F_{ST})/4F_{ST}$), were then calculated.

An assignment analysis was conducted and the origin of each beetle was inferred by the calculation of the likelihood of a specific genotype to be found in a given population according to allele population frequencies using GENODIVE (Paetkae *et al.* 1995; Cornuet *et al.* 1999). Because the migration estimation methods based on *Nm* derivate from F_{ST} can lack precision (Whitlock & McCauley 1999), the assignment method can be a viable alternative to infer migration using Monte Carlo resampling (Cornuet *et al.* 1999; Paetkau *et al.* 2004). Assignment tests were performed on individuals in reference to the location that they were collected. Because the proportion of individuals correctly assigned can be extremely dependent on the number of markers used and the sample size (Benestan *et al.* 2015), we used a subset of 1000 SNPs to perform this analysis. We used replacement rate of 0.005 (Paetkau *et al.* 2004) and Monte Carlo for 5,000 permutations (Cornuet *et al.* 1999) and tested using the likelihood ratio threshold of all 11 locations at 0.05 confidence error.

Population clusters

Population structure was tested using a nested analysis of molecular variance (AMOVA)

using 99,999 permutations with GENODIVE software in two hierarchical levels (Excoffier *et al.* *1992)*. The first hierarchical level tested for differences between crop group regions (rice or wheat) and the second level tested for differences between ecoregions within crop group (Table 1). To visualize the level of introgression between sampled locations and test number of clusters using genetic partitioning we used the software STRUCTURE v2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003) using Bayesian clustering method with a subset of 1,000 random markers using whitelist in STACKS. We used 10,000 burn-in interactions followed by 10,000 Markov chain Monte Carlo (MCMC) steps assuming an admixture model based on individuals and including no prior information on sampling location. We simulated 1-11 populations (k=1 to k=11), replicated 10 times. The most likely number of genetic clusters (K) was estimated using STRUCTURE HARVESTER 0.6.93 (Earl & Von Holdt 2012) and GENODIVE K-mean clustering function (using all 5379 SNPs markers). STRUCTURE output was analyzed using the program CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and visualized using DISTRUCT (Rosenberg 2004).

To estimate the best number of cluster to explain observed variance We used Discriminant Analysis of Principal Components (DAPC) (Jombart 2008; Jombart *et al.* 2010), which optimizes the variance between groups and minimizes variance within groups (Jombart *et al.* 2010). To identify optimum number of clusters to describe the data, we used the DAPC algorithm to compare different numbers of *k* using Bayesian Information Criteria (BIC) to test for the most likely number of clusters to summarize data. First, we transformed the data using Principal Component Analysis (PCA), specifying 200 PCs to be retained (Fig. 5.8A); Subsequently, a BIC value was calculated for each *k* value, and number of cluster determined (*k*) by picking the number of cluster associated with the BIC lowest value (k=6) (Fig. 5.8B). Clusters were determined without sampling location information, and cluster membership

probability was assessed to see if individuals collected at the same place belonged to the same cluster. Further, we analyzed the allele contribution to the PCs. Alleles can contribute differently to the observed variance and therefore might give us additional information about the relative frequency of certain alleles in different locations. All DAPC analyses were performed using *adegenet* package in R.

F_{ST} and IBD

Pairwise population differentiation was estimated using unbiased F_{ST} estimator θ (Weir & Cockerham 1984). We used *RColorBrewer* and *igraph* packages to visualize the F_{ST} relations. We also performed Mantel test with 10,000 permutations to correlate the genetic distances (F_{ST}) matrix with the geographic distance matrix using the libraries *ecodist* (Goslee & Urban 2013) and *ade4* (Dray *et al.* 2010).

Model Selection

We used a model selection approach based on the Akaike's Information Criterion (AIC, Wagenmakers and Farrel 2004) that allows several competing hypotheses to be simultaneously tested to find the single best model or an average of the top models (Johnson & Omland 2004). We selected and tested explanatory variables that we predicted might explain isolation and diversity among collection sites. Because of the large number of variables that could be included in the model and the limited number of observation points, we performed preliminary analysis to identify meaningful variable for comparison. The preliminary screening included geographical variables, crop variables, and grain transport variables. For geographical variables, we tested the relative effect of the average distance to the other locations (distance), latitude, longitude, climate region (temperate, arid, mountain, semi-arid, subtropical), and average temperature at each location. For crop variables, we included crop production information such as the average

wheat or rice acreage within a 50 km radius buffer from each sampling point, obtained clipped from the wheat and rice crop maps using ARGIS ESRI ArcMap v.10.0, and the average yield production in for the state where the samples were collected (NASS 2012). For grain transportation variables, we tested how grain movement by railroads can potentially explain observed variation in genetic parameters. We tested the volume of wheat received from other locations (received), the volume of wheat shipped to other locations (shipped), incoming and outgoing degree for each state, centrality which is the number of shortest paths going through a node in the rail network as a measurement of connectivity (Prater *et al.* 20013; Nopsa *et al.* 2015), and presence or absence of ports at each location as a categorical variable (Denicoff *et al.* 2014).

We standardized response and predictor variables (i.e., converting to *z*-scores) prior to analysis so the beta can be interpreted as the standardized partial regression coefficient or beta weight (Abdi 2004). Beta weights can be comparable and account for the relative contribution of each covariate present in the model. For each response variable, we tested the individual and additive effect of all covariates within group of variables and then between groups of variables comparing the 5 top candidates. We used corrected Akaike's Information Criteria (AICc) to select best candidates, calculate ΔAIC (the difference in AIC with respect to the best candidate), and model probabilities or Akaike weights (w_i) (Johnson & Omland 2004; Wagenmakers & Farrell 2004). We used *glmulti* (Calcagno & Mazancourt 2013) and *MuMIn* (Barton 2009) packages in the R for all modeling analysis.

SNPs under selection

We used the software BAYESCAN v.2.1 to identify candidate loci under natural selection (Foll & Gaggiotti 2008). BAYESCAN uses the F_{ST} coefficient to find candidate loci under selection

based on differences between groups. The F_{ST} calculated for each locus is decomposed into population specific components and loci specific components shared by all populations using logistic regression. The alpha value serves as an indication for selection. Positive values of alpha indicate directional selection, whereas negative values indicate balancing selection. The posterior probability cut-off for outliers used was 0.95 running 100,000 interactions, and 10 was the parameter to 'prior' odd for neutral model.

Results

The average number of sequence reads among the 3 libraries was 222.8 million (Library-1: 233.94, Library-2: 237.7, and Library-3: 225.19) and the average percentage of quality-filtered reads ($\geq$Q30) per library was 91.13% (Library-1: 92.3, Library-2: 90.8, and Library-3: 90.3), giving an average depth of coverage per individual over all SNPs of 35x (Fig. 5.7). Average yield per library was 20.13 GB. Eighteen beetles (7% of the total) had insufficient mean coverage (<5x) and were removed from further analysis. After filtering steps, 45,7018 SNPs were retained before population module in STACKS when it was filtered down to 5,379 SNP markers.

Nucleotide diversity, population history, and migration inferences

There were small, but significant differences in nucleotide diversity among sampled locations. The lowest diversity was in WA and CA, while KS and ND had the highest diversity (Table 5.2). Considering all locations, the observed heterozygosity (H_0) was 0.244 (95% Cl, [0.241, -0.248]), the heterozygosity within populations was 0.280 (95% CI, [0.277, -0.284]), total heterozygosity (H_t) was 0.288 (95% CI [0.284, -0.292]), and inbreeding coefficient (G_{IS}) was 0.133 (95% CI, [0.123, -0.133]) indicating less heterozygotes than expected (Nei 1987). Only North Dakota (2) and Texas (3) had private alleles. The effect of population size can be

estimated by the diversity value divided by an assumed mutation rate. *R. dominica* populations effective size (N_e) ranges ranged from between 54,455 to 60,647. The number of migrants between populations estimated using the F_{ST} values ($Nm= (1-F_{ST})/4F_{ST}$) ranged from between 2.23 (between WA and TX) to and 22.48 (between AR and OK) beetles per generation (Hendrick 2000).

The assignment analysis successfully assigned the majority of beetles to their correct collect location (75%), but there was considerable variation among locations (i.e. 0% LA and 91% CA). Probably due to the sample size, beetles from SC, NK, TX, and CA were more often assigned to their correct location, while LA and one of the KS populations (FKS) did not have any beetles assigned correctly. Two beetles were tagged as possible migrants: one beetle collected at ND was a possible migrant from KS (FKS) and one beetle collected in KS (HKS) was a possible migrant from AR.

Population structure and clustering

The Analysis of Molecular Variance (AMOVA) revealed a significant degree of genetic differentiation among ecoregions (Table 1) within the crop groups ($F_{SC}= 0.023$, P=0.001; Table 3), but not between crops groups of wheat and rice ($F_{CT}= 0.003$, P=0.18; Table 3), using 5,379 SNP markers (Table 5.3).

Genetic differentiation between wheat and rice regions was also not apparent in the STRUCTURE analysis (Figure 5.2). Although a large degree of admixture can be observed between collection regions, clear differentiation can be observed as we increase K. Both STRUCTURE HARVESTER and K-means clustering (lowest value: AIC=79.81, K=2) analysis revealed two clusters. However, K>2 plots show consistent differentiation between locations with variable amount of introgression between them. In contrast to the analysis by STRUCTURE that estimates

number of cluster based on admixture coefficients, DAPC analysis based on allelic variation estimated k=6 as the optimal number of clusters to explain observed allelic variance (Figure 5.3, Figure 5.8). Clusters were formed without sample location information, and had high membership, 89.5% of individuals collected at the same location belongs to the same cluster (Figure 5.3). TX, CA, ND, AR, and OK were the clusters with the highest membership, frequency of individuals collected at the same location and belonging to the same DPCA cluster. We also analyzed the contribution of the alleles to PCA variance and the loading plot indicates that four SNPs reflect most of the variation observed among populations (Figure 5.9).

F_{ST} and IBD

The level of genetic differentiation in pairwise comparisons varied 21-fold between the smallest and the highest degree of differentiation (F_{ST}= 0.004-0.084, Figure 5.4). Without ND, the three KS locations and Ok form the first group of great plains locations; the second branch grouped locations in the coastal plain. TX, and two west coast locations (CA and WA) were the most isolated locations (Figure 5.4). A similar pattern was observed in the network analysis (Figure 5.5). There was significant association between genetic and geographic distances (Mantel's r=0.69, P=0.003) considering all pairwise comparisons (Figure 5.6), and therefore there was evidence for isolation by distance (IBD).

Model selection

For population differentiation (F_{ST}), the top model elected volume of grain received, wheat yield, presence or absence of ports, and geographical distance as best predictors according to AIC_c criteria (Table 5.4). The volume of grain received by a particular location seem to be a consistent predictors and were present in four of top five models. Variable received was more important in explaining variation in population distance (w_i=0.64) than geographic distance

(w_i=0.03). According to the beta weights, volume of grain received is always the most important variable in the models follow by wheat yield and ports. Average model for population differentiation reveals that greater genetic distance is expected to areas that receive greater volume of grain, has lower wheat yield, are more distant, and has ports.

For total heterozygosity (H_t), a measure for diversity, the top model elected volume of grain received, wheat yield, longitude, centrality, and geographical distance as best predictors according to AIC_c criteria (Table 5.4). Again, the volume of grain received by a particular location was a consistent predictors present in four out of five top models. Variable received was more important in explaining diversity (w_i=0.49) than geographic distance (w_i=0.06). According to the beta weights, volume of grain received is always the most important variable in the models follow by wheat yield, longitude, or centrality. Average model for total heterozygosity reveals that greater genetic diversity is expected to areas that receive less grain, has higher wheat yield, are more connected, and nearby other producing locations.

SNPs under selection

In the Bayescan scan for outliers, we found 13 (0.24%) putative candidates under diversifying selection at 0.05 significance level (Figure 5.10). No locus under directional selection was detected.

Discussion

Rhyzopertha dominica populations have shown significant structure that was reflected in different degrees of isolation and genetic diversity. By using thousands of markers, we examined and extract genetic diversity information from local populations and successfully found correlation with geographic patterns and with information about the agriculture system and the insects' biology (Coop *et al.* 2010; Giska *et al.* 2015). Here, we have found small but significant

differences in nucleotide diversity among populations that was positively correlated with the population effective size (N_e) and negatively correlated with F_{ST} values calculated for each location. An interesting finding is that the largest and most diverse populations are also located in places with the largest grain production in the United States (NASS 2012).

All populations have shown lower values of observed heterozygous than expected, the inbreeding coefficient (G_{IS}) did not correlate with nucleotide diversity, N_e, nor F_{ST}. Similar low heterozygosity has been described in the literature for other stored product insects (Demuth *et al.* 2007; Drury *et al.* 2009) and for other beetle species (Brouat *et al.* 2003; Schrey *et al.* 2008). Heterozygosity deficiency may be associated with the way this species colonizes areas and disperses from them. For instance, outdoor trap capture has shown that flying females are often mated; thus, when infesting a large mass of grain such as a grain elevator or a grain bin, it behaves much like a colonizing propagule. It is possible that the founding event that starts an infestation leads to a more inbred population (Wade & McCauley 1988). This effect can be substantial if the colonizing propagules are coming from a single population or a small group of relatives (kin-structured colonization; Wade and McCauley 1988; Whitlock and McCauley 1990; Wade et al. 1994, Drury et al. 2009). Those immigrants are likely to share higher levels of genetic similarity among themselves and if more than one colonizing propagules infest a grain mass coming from different breeding sites, they will exhibit a higher level of genetic differentiation from one another as was observed in *Tribolium* populations (Drury et al. 2009). The reunion of dispersing inbreed colonizers can temporarily create substructure in the local population (Wade & McCauley 1988; Whitlock & McCauley 1990; Zhivotovsky 2015) that is like to disappear within few generations of random mating (Berthier *et al.* 2006).

Outside storage traps might capture different patterns depending on the local dynamic, in example, very different individuals flying in (colonization) from multiple locations or very similar individuals flying out (dispersing). We can evaluate heterozygous deficiency, diversity, isolation and infer about pattern of population expansion and retraction as well as colonization and substructure in *R. dominica*. Heterozygous deficiency is not the only pattern that has been found in stored product insects, other species have shown excess of heterozygous that was attributed to bottlenecks caused by chemical (i.e., fumigation) or heat control (Fields & White 2002; Semeao *et al.* 2012; Coelho-Bortolo *et al.* 2016; Blanc 2006). Nonetheless, we have to be careful interpreting and generalizing inbreeding coefficients such as G_{IS} due to fact that they are more related to properties of the mating system within population rather than evolutionary processes that lead to divergence among populations such as F_{ST} coefficients (Holsinger & Weir 2009).

Population sub-division

Rhyzopertha dominica is widely distributed across US; however, this distribution is not uniform throughout their range, and we expected to encounter different degrees of isolation (Ellstrand & Elam 1993). Physical barriers such the Rocky Mountains and deserts, historic processes of colonization, and characteristics of the life history (i.e., dispersal behavior) have impact on both gene flow and consequently on population structure (Lowe & Allendorf 2010). We have found a significant degree of isolation in populations from California, Washington, and Texas, and because those locations have also the largest average distance to other locations, isolation by distance (IBD) can be implied. Other studies on stored product pest have found significant degree of structure but lack of IBD (Bas et al. 2000; Drury *et al.* 2009; Semeao *et al.* 2012; Coelho-Bortolo *et al.* 2016; Thagaraj *et al.* 2016), which led to the prevalent hypothesis

that human-aided movement was a more important factor than flight dispersal in defining population structure (Drury et al. 2009). Here we found that geographic distance can play a significant role on population structure even in stored product insects with high degree of predicted anthropogenic movement. Distance can be considered an important factor determining structure of stored product pests. The greatest number of possible migrant would come from Arkansas according to *Nm* methodology; however, a great number of assumptions are necessary for *Nm* interpretation such as the geographical structure, the equal population size and rates immigration and emigration among subpopulations, the absence of natural selection (Wright 1951). Assignment analysis, on the other hand, tagged one insect from Arkansas and one from Kansas as possible immigrants, which is not an unrealistic expectation.

Data presented here demonstrate that *R. dominica* populations experience a considerable amount of gene flow that was reflected in the high degree of population admixture and illustrated in the structure plots and by the relatively low number of private alleles found. According to STRUCTURE analysis, the likely number of population groups was two, California and Texas were grouped together while all the other location were in a second group. However, a much larger number of groups can be differentiated as we increase K value. When number of clusters was inferred by allelic frequency through DAPC, we found that six clusters was the optimum number to explain allelic variance in the principal components. The distribution of individuals from the genetic clusters does not match exactly our ten proposed ecoregions, but there was considerable overlap. The hierarchical analysis of molecular variance confirmed the trend showing significant structure caused by the ecoregions, but not for the crop type. Ecoregions are a more natural way to see boundaries and limits rather the arbitrary lines that divides states. The F_{ST} values also demonstrated that in general locations that belong to similar habitats and are geographically

close are less differentiated among themselves. The three Kansas locations fell in the same branch and the next closest location was Oklahoma, which has similar habitat (i.e., belong to the same domain). That is also evident when we test isolation by distance correlating geographic distance and genetic distance. However, Texas does not fit completely in that model. For instance, Texas is very close geographically from Louisiana, but seemed to be very distant genetically.

Crop vs ecoregions

We did not find significant structure caused by crop region (i.e., 1.25% of the total variance was explained by crop type). However, we cannot be conclusive regarding this question because only the region in Texas where are traps were placed is an exclusively rice production region according to our classification (crop type within 50 Km radius). Although predominantly rice regions, the locations in AR and LA also have some wheat grown within the established perimeter, making them actually mixed crop regions. Texas presented a substantial degree of differentiation compared to a close location such as LA and TX was also one of the only two regions that had private alleles and was responsible for high allelic variation in the DAPC along with ND. So this suggests that there may be a crop effect that was not detectable using our sample locations. The hypothesis that crop type could be a factor causing population structure has been investigated in the past in wheat and rice mills for *Tribolium* (Semeao *et al.* 2012), but in that study the variation within commodity type or region grouping was equal to or greater than that between groups, showing just weak relation. A finer scale sampling effort including more locations that are exclusively rice and wheat is necessary to answer the question whether crop type can be an important factor structuring populations.

In DAPC, we analyzed allelic contribution to the PCA to identify which loci are accounted for most of the allelic variation. We have found that five loci present great differences in frequency. Genetic differentiation can occur in multiple ways, including both neutral differentiation in the absence of gene flow and adaptive differentiation in response to selection in different environments (Charlesworth *et al.* 1987; Drury et al. 2009). To this point we can only assume the observed allelic variation is due to local adaptation driven by specific requirements of the habitat. The Bayerscan analysis found thirteen loci under selection. Divergent selection in two different environments (i.e., TX and ND) may cause genetic differentiation by eliminating an allele from one population while fixing it in another population in the other environment. We are currently mapping those candidates into the reference genome and proceeding further investigation on the potential role on local adaptations.

Source-sink dynamics

Because distance has not been found as a major factor affecting population structure in stored product insects, grain transportation is often evoked as the best explanation for the lack of IBD (Ryne & Bensch 2008, Bas et al 200; Blac et al. 2006). In the present work, we have found significant correlation between geographic distance (km) and genetic distance ($F_{ST}/1-F_{ST}$) using Mantel test; however, the high rate of admixture (Fig 2) and the correlation between nucleotide diversity and wheat yield suggest that agriculture activity and grain transportation are also important in impacting genetic parameters of the tested populations. Model selection allows us to test several competing hypotheses by weighting and establishing relationships between variables (Johnson & Omland 2004). We used geographic, crop information, and transportation variables to assess the relative importance of each covariant explaining genetic isolation and diversity. The prediction was that larger geographic range over which grain is grown and shipped should be

associated with greater genetic diversity and less geographic isolation (source population) (Semeao *et al.* 2012), and places that tend to receive more grain will have less diversity and are more isolated (sink population). Sink populations diversity might vary according to the population size, number of migrants, and number of different source populations connected to it, which might not necessarily mean sink population will have higher diversity. Our predictions were confirmed and the covariant volume of grain received was elected as the most important in all models evaluated. This variable defines whether a location is a sink or a source (i.e, low values of grain received is associated with source population and high values of grain received is associated with sink population). Acreage of planted wheat, presence of port, and distance were also selected to the top five models to explain population differentiation (i.e., average F_{ST}). Instead of the presence of port, centrality was more important to explain diversity (i.e., total heterozygosity). The average model for population differentiation predicts that more differentiation is expected in places that receive substantial amounts of wheat (sink populations), have lower wheat production, are distant, and have ports for international shipments. On the other hand, more diversity is expected in places that do not receive substantial amount of grain (source populations), are great producers of wheat, are highly interconnected by railroads, and do not have ports.

Conclusions

Here we have shown how features of the environment in which local populations are present, agriculture management, as well as storage and transportation of commodity are likely to affect different aspect of an insect pest population. Inbreeding coefficients gave us insights on how populations infest and develop within a storage sites; however, hererozygosity deficiency observed needs further evaluation to determine if it is due to sample bias, such as timing before

or after fumigation or being nearer to stored grain vs natural habitat locations, or if it truly represents a pattern of substructure or kin-structure. The most diverse populations were located in most regions with greatest wheat productivity. Large production areas offer more opportunities for *R. dominica* to breed and these regions can potentially can be a source of migrants to other less productive locations. The displacement from one location to another perhaps by flight, or by human-aided means using railroads connecting local populations or ships and ports connecting populations globally, can largely explain population differentiation patterns in *R. dominica* in the United States.

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Figures and Tables

Table 5.1 Sampling location information on ecoregion, crop type and number of individuals successfully genotyped (N_{GEN}).

Ecoregions	Locations	Code	Latitude	Longitude	Crop	N_{GEN}
South Central Plains	Alexandria-LA	LA	31.2928	-92.4592	Rice	7
Western Gulf Coastal Plain	Beaumont-Texas	TX	30.08	-94.1267	Rice	29
Flint Hills	Manhattan-Kansas	MKS	39.1917	-96.5917	Wheat	7
South central semiarid	Hudson-Kansas	HKS	38.0608	-97.9297	Wheat	11
Western Corn Belt Plains	Fairview-Kansas	FKS	39.84	-95.7275	Wheat	5
Central California Valley	Parlier-California	CA	36.6117	-119.526	Wheat	42
Mississippi Valley Loess Plains	Arkansas	AR	35.8281	-90.6942	Rice	30
South central semiarid	Stillwater-Oklahoma	OK	36.1157	-97.0586	Wheat	11
Lake Agassiz Plain	Fargo-North Dakota	ND	46.8772	-96.7894	Wheat	43
Southeastern Plains	Orangeburg-South Caroline	SC	33.4969	-80.8622	Wheat	21
Columbia Plateau	Spokane- Washington	WA	47.6589	-117.425	Wheat	4

Table 5.2 Genetic statistic of *Rhyzopertha dominica* 9 United States locations estimated from RADseq data (209 individuals and 5379 loci included) for all nucleotide position; site polymorphic, H_0 observed heterozygosity, H_E expected heterozygosity, nucleotide diversity (π) (mean±SE).

Sample location	Sites polymorphic	H_0	H_E	G_{IS}	π
Louisiana	0.9969	0. 249	0.282	0.115	0.0047±0.0001
Texas	0.9969	0.244	0.272	0.101	0.0045±0.0001
Kansas	0.9966	0.238	0.287	0.171	0.0049±0.0001
California	0.9972	0.236	0.277	0.149	0.0041±0.0001
Arkansas	0.9968	0.244	0.285	0.144	0.0047±0.0001
Oklahoma	0.9968	0.244	0.285	0.143	0.0047±0.0001
North Dakota	0.9967	0.246	0.282	0.130	0.0048±0.0001
South Caroline	0.9968	0.248	0.278	0.107	0.0047±0.0001
Washington	0.9971	0.238	0.265	0.103	0.0044±0.0001

* Sites polymorphic and diversity estimates refer to all positions (variant and fixed)

* Kansas combines the three sampled locations (HKS, MKS, FKS)

Table 5.3 F_{ST} variance within populations, F_{SC} variance among populations within group, F_{CT} variance among groups

Source of variation	Variation (%)	F_{ST}	F_{SC}	F_{CT}
Among crop-groups	1.25			0.003
Among ecoregions within crop-groups	10.23		0.023**	
Within ecoregions	88.52	0.199**		

Table 5.4 Model selection results for top five models and average model for response variable: population differentiation (F_{ST}) and heterozygosity (H_t). Beta weights are presented with selected variables and represent the weight of each variable. Best models are those with the lower AIC value. The delta Akaike and Akaike's weights are also shown.

Competing models	AICc	ΔAICc	w_i *(AIC)*
Population Differentiation (F_{ST}) =			
1) 0.9 Received	21.35	0	0.64
2) 0.9 Received - 0.3 Yield	23.48	2.13	0.22
3) 1.5 Received - 0.7 Yield - 0.6 Ports	25.96	4.61	0.06
4) 0.7 Received + 0.3 Distance	27.22	5.87	0.03
5) 0.8 Distance	27.24	5.89	0.03

Average model: F_{ST} = 0.9 Received - 0.4 Yield+ 0.5 Distance - 0.6 Ports

Competing models	AICc	ΔAICc	w_i *(AIC)*
Total Heterozygosity (H_t) =			
1) - 0.8 Received	22.32	0	0.49
2) - 0.9 Received + 0.3 Yield	23.85	1.53	0.23
3) - 0.7 Received – 0.5 Longitude	24.89	2.57	0.14
4) - 0.7 Received + 0.3 Centrality	25.85	3.53	0.08
5) - 0.8 Distance	26.59	4.27	0.06

Average model: H_t = - 0.9 Received + 0.3 Yield + 0.3 Centrality - 0.6 Longitude - 0.1 Ports

Figure 5.1 *R. dominica* sampling locations in the United Stated according to (A) ecoregions (legend refer only to ecoregions sampled according to EPA classification) and (B) wheat (green) or rice (blue) crop region. Map displaying ranges of all wheat and rice harvested for grain as a percent (%) of harvested cropland in 2012. Darker colors indicate a higher percent of the cropland acreage as all wheat and rice harvested for grain (source: NASS 2012).
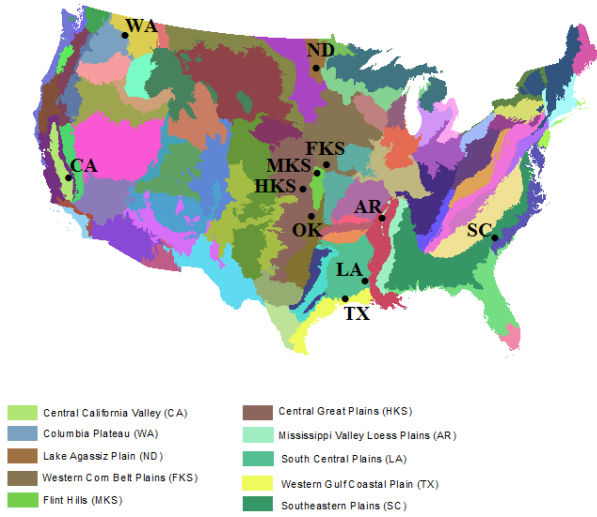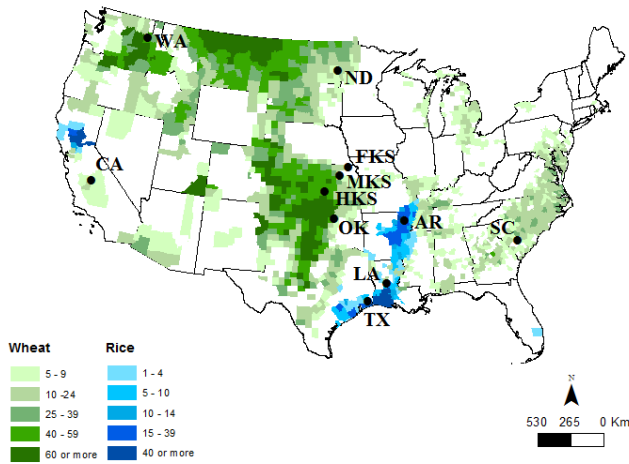
Figure 5.2 Model-based clustering of 210 beetles collected in 11 locations in the United States using 1,000 SNPs markers. Analysis was performed using the program STRUCTURE, with K representing the assumed number of putative populations (HKS, FKS, and MKS are shown as KS). (STRUCTURE HARVESTER predicts K=2 and DPAC predicts K=6)
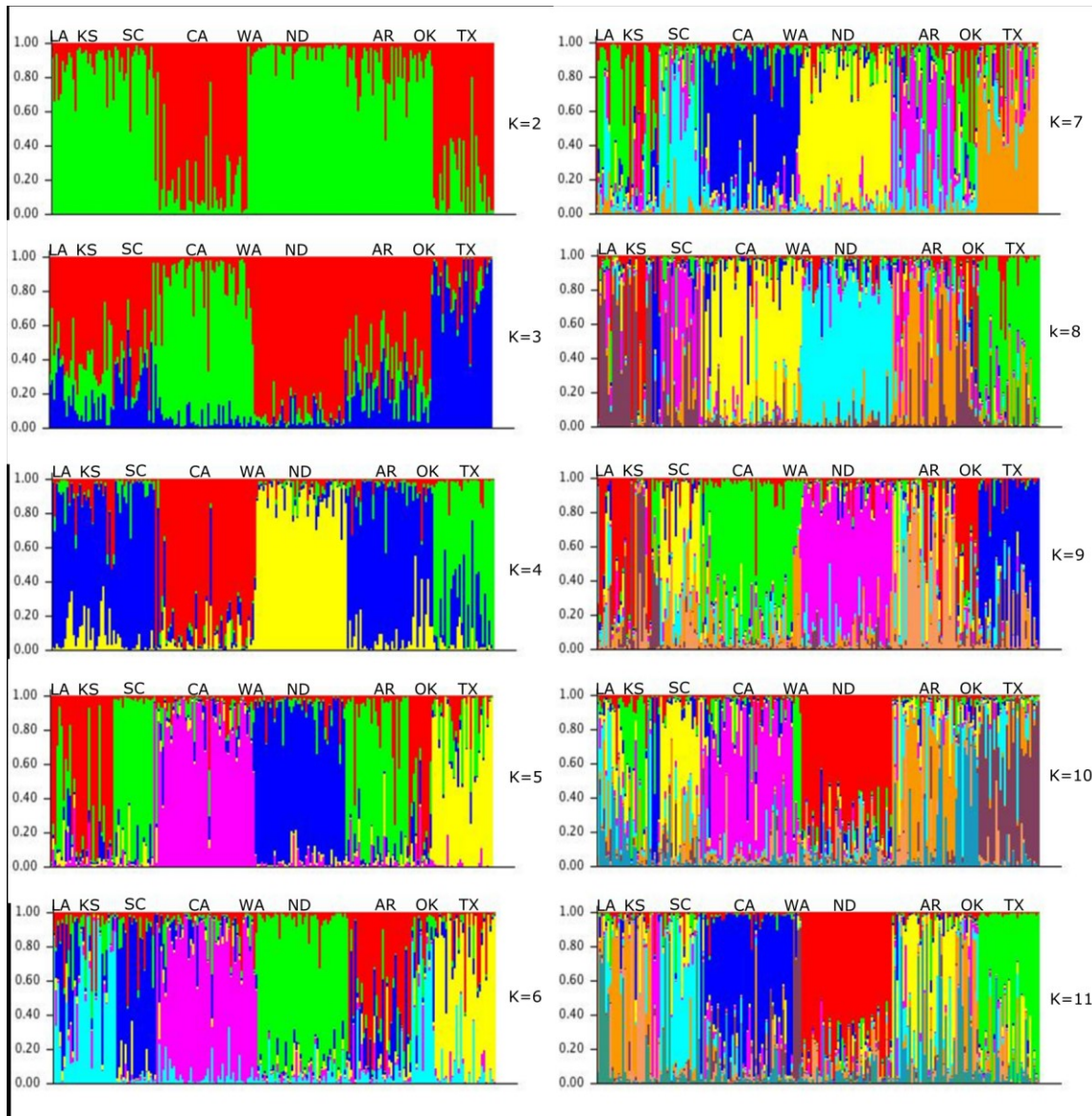
Figure 5.3 DAPC clustering of 209 individuals from 11 locations in the United States using 5,379 SNPs markers (K=6). (A) The plot of the two components combination revealing of differentiation between cluster (colors represented) without sampling location information. (B) Plotting the densities of individuals on the discriminant function with different colors for DAPC clusters.

(A)
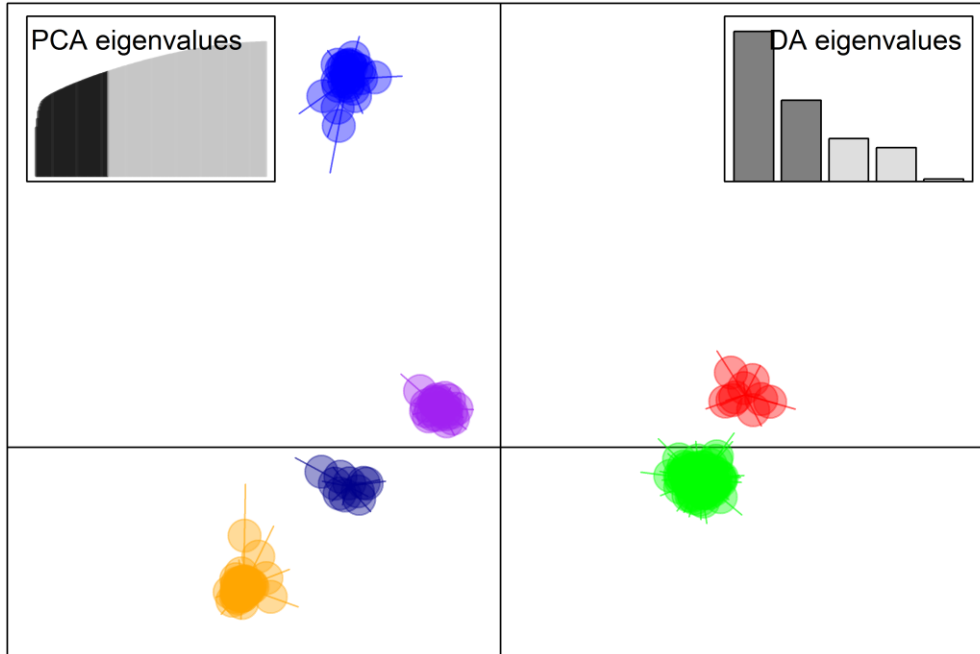
(B)
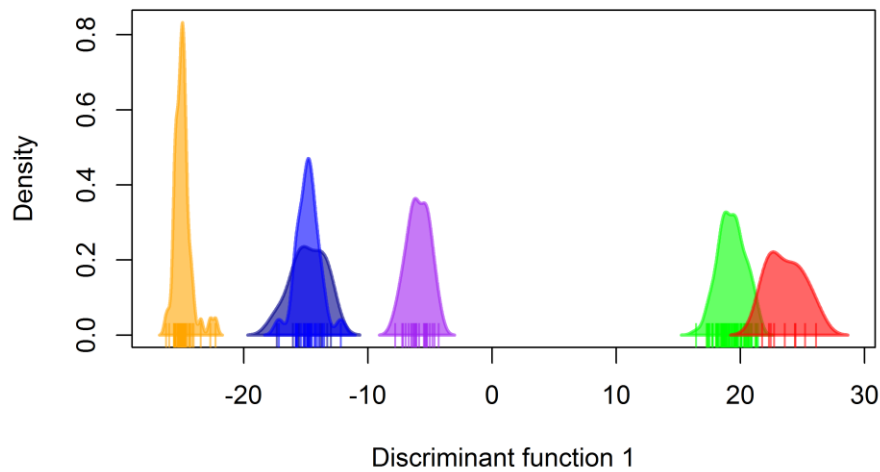
Figure 5.4 F_{ST} population dendogram and heatmap based on F_{ST} values among the 11 *R. dominica* sampling locations. Darker color represents greater degree of differentiation.
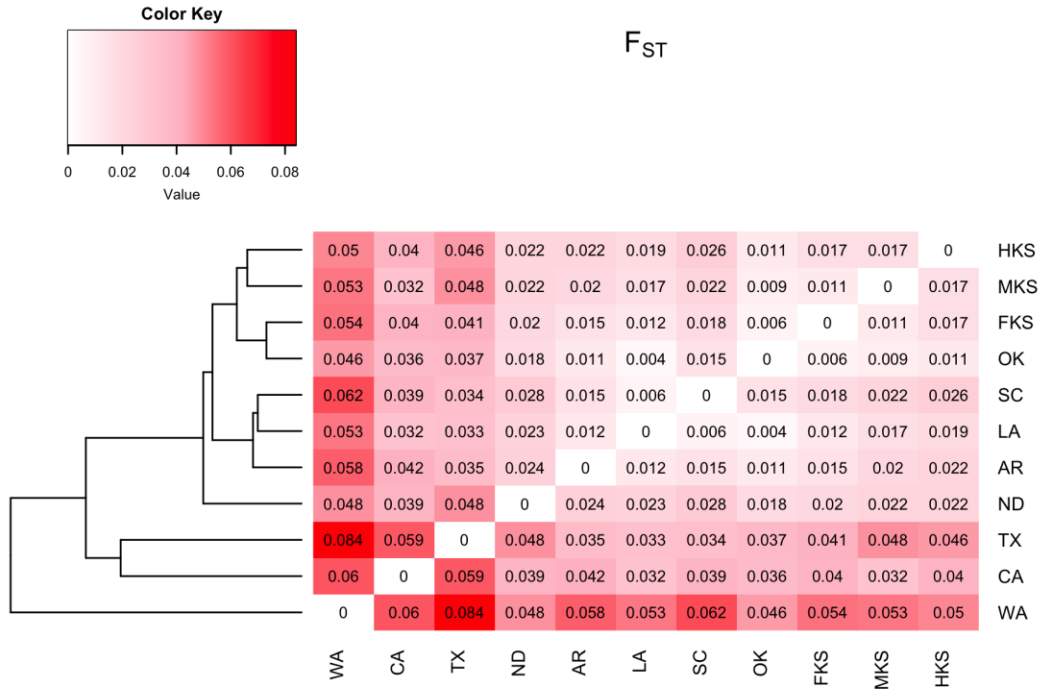
Figure 5.5 Network showing F_{ST} distance (links), wheat production (node size), and role as source (green) or sink (red) according to rail transport of wheat in the United States. Source locations are greater producers and tended be less isolated. Sink location are small producers and tented to be more isolated.
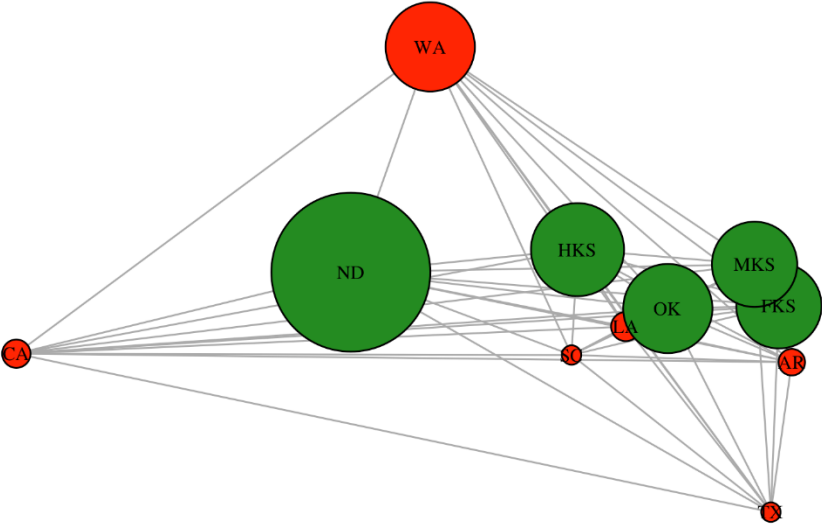
Figure 5.6 Isolation by distance (IBD) of *R. dominica* population based upon correlation between genetic distance ($F_{ST}/1-F_{ST}$) and the geographic distance (Km) (Mantel's r=0.69; P=0.003).
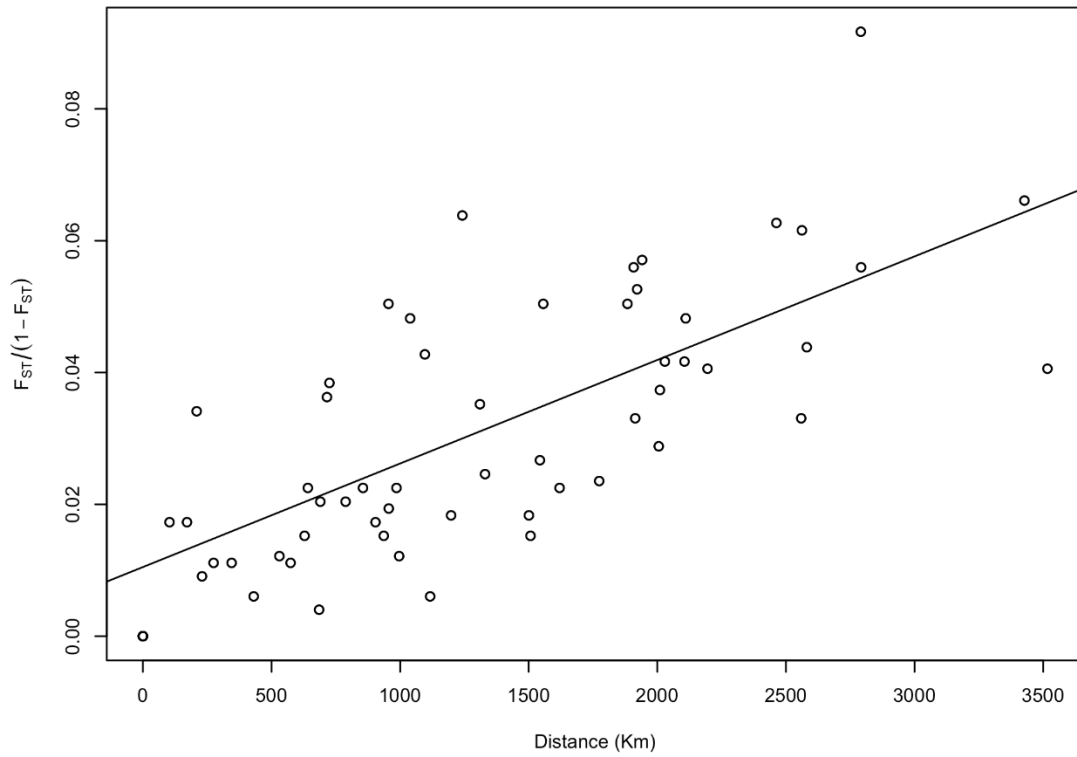
Supporting information

Figure 5.7 Depth of coverage (DP) *per Rhyzopertha dominica* beetle after STACKS population module filtering for minimum 10x coverage. Average coverage per individual (ID in the x axis) is 35x coverage.
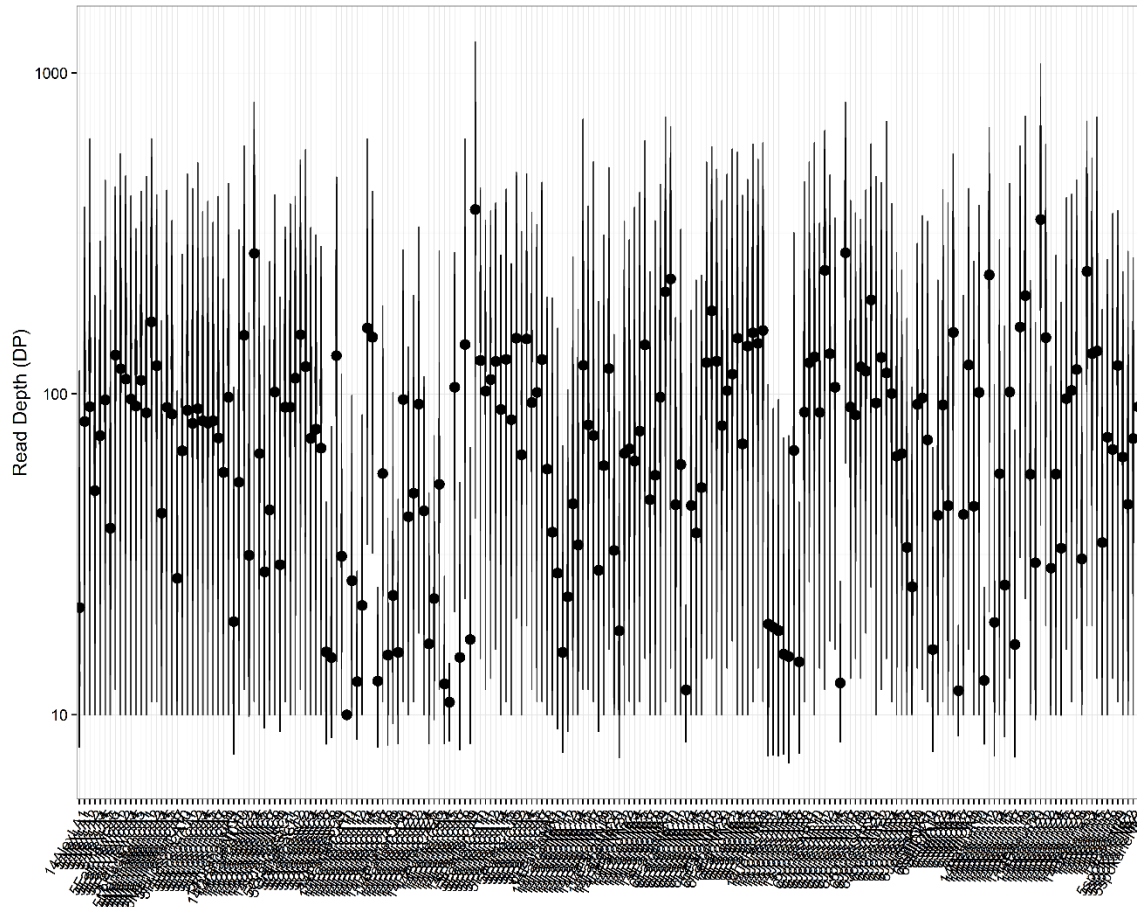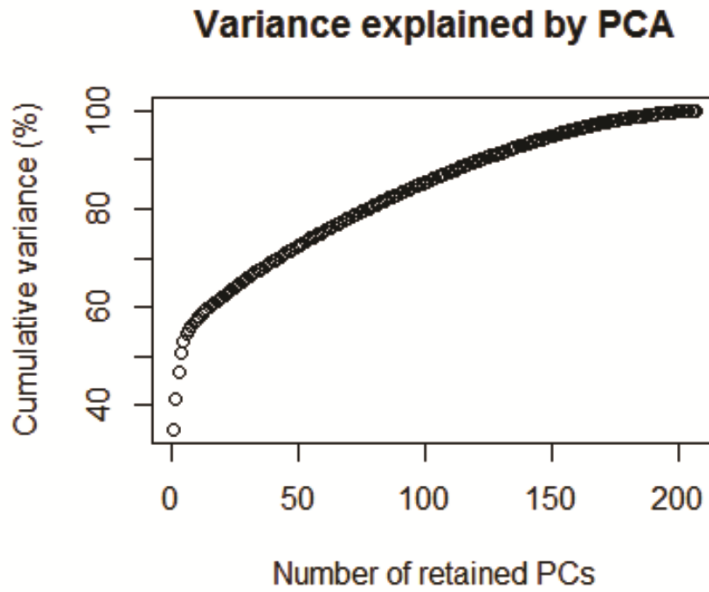
Figure 5.8 Genetic diversity using multivariate DAPC analysis. (A) The function shows cumulated variance explained by the eigenvalues of the PCA. (B) The function shows BIC values for increasing *k* value. Lower BIC value (*k*=6) indicates clusters should be retained.
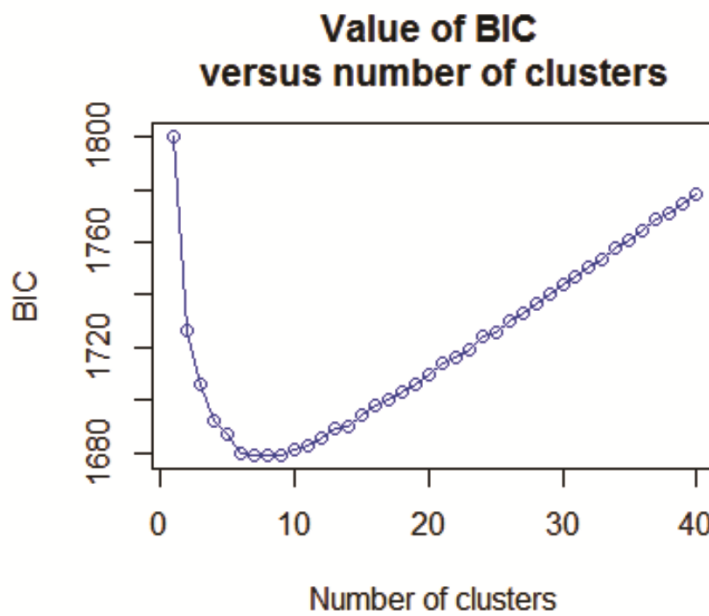
(A)

(B)

Figure 5.9 Loading plot from DAPC of *R. dominica* from 11 different locations in the United States showing the contribution of alleles to the first DAPC eigenvalue. The height of each bar is proportional to the contribution of the corresponding allele to the first component of the analysis. Only alleles whose contribution is above the arbitrary threshold (grey horizontal line) are indicated. Alleles are labeled according to its stack ID number.
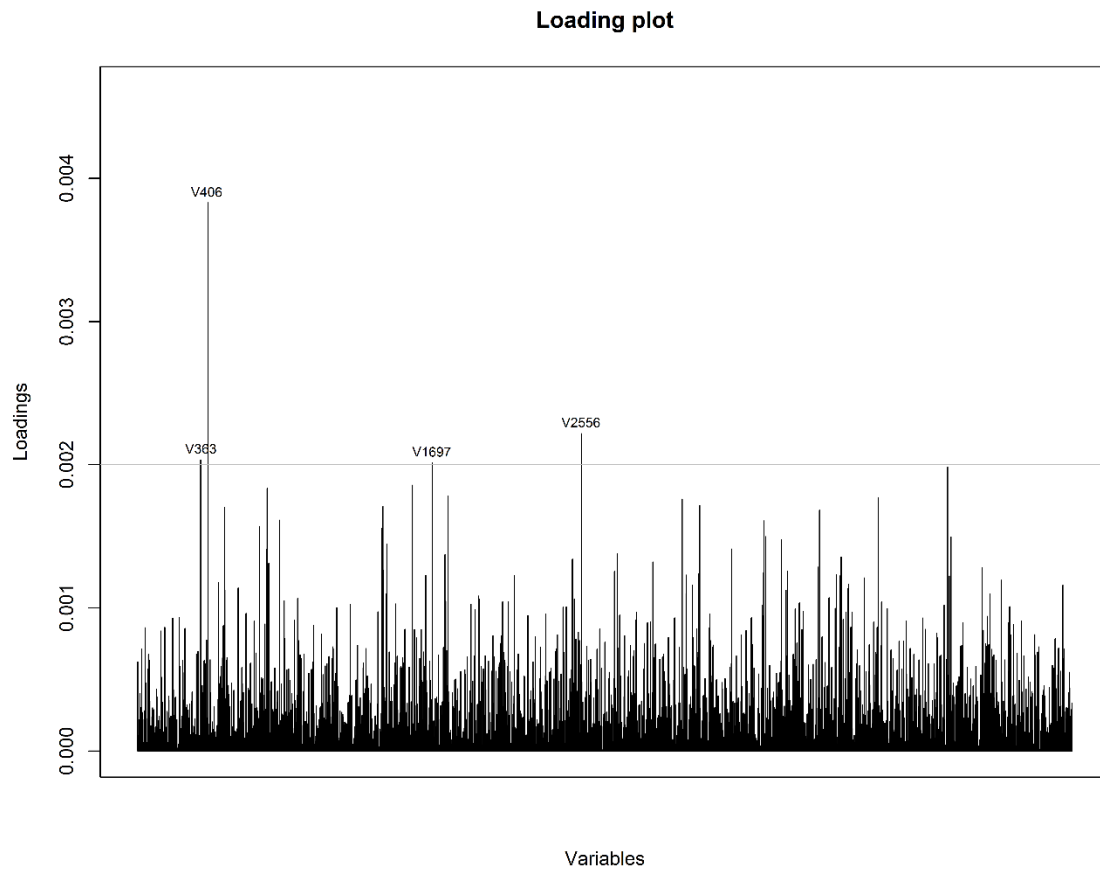
Figure 5.10 Signature of natural selection in United State *Rhyzopertha domininic*a populations using the program Bayescan. The vertical axis indicates mean F_{ST} values between each of the 11 locations, and the horizontal axis indicates the logarithm of the q value. The vertical line indicates the $\log_{10}$(q-value) corresponding to the false discovery rate threshold of 0.05 loci. Number represents loci ID and the ones on the right of this line are putatively under selection.