INFLUENCE OF LANDSCAPE STRUCTURE ON MOVEMENT BEHAVIOR AND HABITAT USE BY RED FLOUR BEETLE (*TRIBOLIUM CASTANEUM*)

by

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B.S., College of Mount Saint Joseph, 1996
M.S., Miami University, 1999

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Entomology
College of Agriculture

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Abstract

Theoretical and empirical ecological research has emphasized the need for understanding how animals perceive and respond to landscape structure and the importance of integrating both behavioral and landscape approaches when studying movement behavior. Knowledge of insect movement behavior is essential for understanding and modeling dispersal and population structure and developing biologically-based integrated pest management programs. My dissertation research addresses questions concerning how insects respond to landscape structure by examining movement behavior of an important stored-product pest, red flour beetle (*Tribolium castaneum*), in experimental landscapes.

Results show that beetles modify movement behavior depending on landscape structure. Edge effects and interpatch distances may influence landscape viscosity, or the degree to which landscape structure facilitates or impedes movement, resulting in significant differences in velocity and tortuosity (amount of turning) of movement pathways, as well as retention time in landscapes with different levels of habitat abundance and aggregation. Perceptual range, or the distance from which habitat is detected, appears to be limited while beetles are moving in a landscape as they did not respond to a flour resource before physical encounter. Beetles showed differential responses to patches with various characteristics, entering covered patches more quickly than uncovered patches with more resource or the same amount of resource. Permeability of patches changed with subsequent encounters suggesting that full evaluation of patch quality may only occur after entering a patch. Beetles responded to landscape structure differently depending on the activity in which they were engaged. Distribution of movement pathways was similar to that of the habitat, but distribution of oviposition sites were significantly more aggregated than pathways and habitat. Oviposition site choice may be influenced by a complex set of factors which include previous visitation, amount of resource, travel costs, and edge effects. Insights were gained concerning how red flour beetle perceives resources, modifies search strategies, responds to boundaries, and chooses reproductive sites in patchy landscapes. This research provides new information regarding how red flour beetle interacts with landscape structure that has implications in the areas of behavioral and landscape ecology and applications in stored-product insect ecology.

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Table of Contents

List of Figures

List of Tables

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CHAPTER 1 - Introduction

Insects that attack stored products cause considerable damage to bulk grain and processed commodities worldwide (Hagstrum and Flinn 1995). Damage to bulk stored agricultural products alone is estimated to be between $1.25 and 2.5 billion a year (Scholler et al. 2005) in the United States. Chemical pesticides have been the most widely used method to prevent economic loss caused by stored-product pests. Because of potential hazards to human health and the environment, however, these compounds are becoming more and more restricted in their use. Protection of stored-products has now shifted from using mainly conventional pesticides to using integrated pest management (IPM), which employs a variety of control methods. Many of these alternative measures require a better understanding of the biology, behavior, and ecology of stored-product insects.

Stored-product pest behavior and ecology has been extensively studied in bulk stored grain (Sinha 1991, Hagstrum et al. 1995), but information is limited on pests in spatially- and temporally-variable ecosystems such as food processing plants, warehouses, and retail stores. Stored-product environments can be thought of as landscapes consisting of a mosaic of favorable and unfavorable patches in which insects may have a patchy distribution over space and time. Probabilities that insects within patchy landscapes will be killed or disturbed by pest control measures differ. Successful dispersal from one patch to another drives the ability of stored-product insect populations to persist, thereby challenging the efficacy of control tactics (Campbell et al. 2004). There has been an increase in both theoretical and empirical studies of the influence of landscape structure on movement of insects (Wiens and Milne 1989, Crist 1992, Johnson and Milne 1992, Wiens et al. 1997, With et al. 1997, With et al. 1999). However, there are few studies that have focused on stored-product insects (Campbell et al. 2002, Morales and

Ellner 2002) even though these insects are frequently found in landscapes with patchily distributed resources.

The red flour beetle (*Tribolium castaneum*) Herbst is a cosmopolitan, stored-product insect pest that is responsible for millions of dollars of economic damage to a wide range of stored and processed foods. Red flour beetles are well-adapted to finding and exploiting resources located in patchy landscapes and persisting on small amounts of food accumulated in refugia (Campbell and Hagstrum 2002). These characteristics contribute significantly to their pest status. Knowledge of movement behavior of insects in patchy landscapes is essential for understanding their distribution and movement patterns, modeling dispersal and population structure, and developing biologically- and biodiversity-based integrated pest management programs.

The red flour beetle

The red flour beetle is a major, worldwide pest of stored grain, cereal products, and many other dried and stored commodities used for human consumption. These beetles are thought to have originated in India (Hinton 1948) where they live under tree bark and in rotten logs, and feed as scavengers on a variety of plant and animal matter, including insect eggs and pupae (Sokoloff 1974). Although this pest readily invades many commodities, milled grain products such as flour are the preferred foods (Good 1936). Red flour beetles have a long history of association with humans and their structures such as warehouses, mills, food-processing facilities, retail stores and homes. For a red flour beetle, these anthropogenic structures constitute patchy landscapes where food, breeding, and oviposition sites are found mainly in small refugia such as cracks, crevices, and parts of machinery where food material accumulates (Campbell and Hagstrum 2002). These resource patches can vary considerably in their size and persistence, and thus in their quality as a resource. Patches, even as small as 0.005 g, can provide habitat for reproduction in red flour beetles (Campbell and Runnion 2003). At any given time, only a portion of these patches may be occupied by the insects and the probabilities that insects within these spatially separated patches will be killed or disturbed by pest control measures differ. Because of the patchy nature of both the non-anthropogenic and anthropogenic landscapes exploited by red flour beetle, they have evolved an excellent dispersal response, which contributes to population persistence and overall fitness. This same trait, however, poses serious challenges for developing effective pest management tactics (Campbell and Hagstrum 2002).

Described as a primary colonist and a refuge species, red flour beetles disperse readily throughout the adult stage (Ziegler 1976, Lavie and Ritte 1978), prefer habitats without previous insect infestation, and may scatter eggs among many small patches that often are rapidly depleted (Campbell and Runnion 2003). Past work on the subject of dispersal in *Tribolium* has focused mainly on movement within bulk grain (Surtees 1963, Hagstrum and Leach 1972) or emigration from patches of flour (Hagstrum and Gilbert 1976, Ziegler 1976, Lavie and Ritte 1978, Korona 1991). Several factors influencing emigration in *Tribolium* have been extensively studied, including density (Naylor 1961, Hagstrum and Gilbert 1976, Ziegler 1978), age (Hagstrum and Gilbert 1976, Ziegler 1976), and food quality (Ogden 1970b, Ziegler 1978). Red flour beetle dispersal has also been investigated from an evolutionary standpoint on topics such as fitness consequences (Ogden 1970a, Ziegler 1976), genetic determinants of dispersiveness (Ogden 1970a, Ritte and Lavie 1977, Riddle and Dawson 1983, Korona 1991), and the relationship between dispersal and life-history traits (Lavie and Ritte 1978, 1980, Ben-Shlomo et al. 1991). Information on red flour beetle movement outside of food patches is very limited because early studies focused only on emigration from patches (Korona 1991) or because the experimental design constrained movement to artificial channels between patches (Naylor 1961, Lomnicki and Krawczyk 1980, Ben-Shlomo et al. 1991).

Many factors may influence the patch selection process and only a few have been examined in red flour beetle. Beetles have been shown to respond to food volatiles (Seifelnasr et al. 1982, Barrer 1983, Phillips et al. 1993) and aggregation pheromone (Oben-Ofori 1991). Campbell and Runnion (2003) investigated the ability of female *T. castaneum* to maximize fitness by adjusting clutch size in food patches differing in size. They found that females laid more eggs in larger amounts of flour and that the number of eggs was consistent with that predicted to be optimal for the amount of resource (i.e., Lack clutch size) (Lack 1947). Several recent studies of *Tribolium* dispersal have taken a landscape perspective, focusing on movement and behavior in response to various substrates (Morales and Ellner 2002) and the spatial pattern of resource patches (Campbell and Hagstrum 2002). Morales and Ellner (2002) emphasized that behavioral heterogeneity between and within individuals should be incorporated into correlated random walk models in order to more accurately predict spatial spread of *T. confusum.* Campbell and Hagstrum (2002) observed that beetles were often found inactive outside of food patches and moving along the structural edge of the experimental arena, suggesting that patches near structural edges may have higher probabilities of infestation than those away from such edges. In a study conducted in simulated warehouses, Toews and collaborators (Toews et al. 2005a) found that pheromone trap catches of *T. castaneum* were greater in traps placed in corners, along walls, and near food patches, suggesting that beetles may avoid the centers of warehouses and food-processing facilities. Results of another study in the same simulated warehouses demonstrated that pheromone traps placed near food patches captured more larvae and adults and concluded that this information should be considered when planning targeted insecticide applications (Toews et al. 2005b). A study using trap-capture data in a commercial food-processing facility determined that the distribution of *T. castaneum* was temporally and spatially patchy (Campbell et al. 2002). Contour-mapping revealed foci of high trap catches that were present throughout the sampling period, but other areas of high trap capture were variable over time. At all sampling dates red flour beetle had a clumped distribution except after an insecticide treatment. The highly variable and patchy nature of the distribution of red flour beetle in this facility raises questions concerning their movement. For instance, what is the size of the area in which they are active and what is the maximum distance patches may be connected by dispersal? Results of these studies highlight the need for additional detailed investigation of red flour beetle movement behavior in response to landscapes in which patches vary in size, pattern, and quality.

Movement behavior and landscape structure

Movement behavior of individuals across heterogeneous landscapes impacts many important ecological phenomena such as patch resource use, population spread, and metapopulation dynamics (Kareiva 1990, Bell 1991, Turchin 1991, 1993). The term "landscape", as used by ecologists, is usually defined in one of three ways: (1) as an expansive land area in which many populations live (Forman and Godron 1986); (2) as an organizational level in an ecological hierarchy that is placed between biosphere and ecosystem (Allen and Hoekstra 1992); and (3) as an area of any size composed of a spatial mosaic (With 1994a). Here, I take the third view and consider a "landscape" to be a heterogeneous area of any size.

Suitable habitats are usually embedded in a matrix of unsuitable or inhospitable areas, resulting in a mosaic of patches that differ in their quality and usefulness. The spatial mosaic of the landscape and the scale at which this pattern is perceived by an organism influences movement behavior (Kotliar and Wiens 1990, With 1994b) and ultimately the ability of animals to find resources for shelter, food and reproduction (O'Neill et al. 1988, Gardner et al. 1993, Pearson 1996). Resources on landscapes can be patterned in different ways: uniformly, randomly, or clumped. The ability to locate these resources is influenced not only by the pattern, but also by the amount of habitat fragmentation, i.e., the size of habitat patches and the distance among them (Doak et al. 1992, With and Crist 1995, Pearson 1996).

How an organism responds to the pattern of habitat is a central question in ecology (Wiens 1989, Levin 1992). At what spatial scale does an animal perceive the landscape as patchy? Are small habitat fragments within a specific area perceived as separate patches or are they perceived as individual resource concentrations within one large patch that encompasses the entire area being examined? Answering these types of questions is important for understanding how organisms interact with landscapes. Patterns of resource use are scale-dependent (O'Neill et al. 1988) because the grain (smallest area) and extent (largest area) of search behavior depends on the spatial pattern of resources, the perception of the individual, and the search strategy it uses. If food or habitat patches are uniformly distributed on the landscape within the animal's dispersal range, search effort will likely be minimized and the animal will spend less time and energy searching. If resources are patchy on the landscape and beyond the distance at which the animal may detect them, then search effort may need to be expanded and more time and energy will be spent searching. Searching behavior may be very different on a fine-grained landscapes with small patches that are close together versus one in which habitat patches are large and clumped (Bell 1991).

How should an organism move through landscapes with different spatial arrangements of habitat? What is the best search strategy for acquiring food, shelter, and mates in an often unknown and dangerous environment? Searching animals usually experience an elevated risk of mortality and, according to foraging theory, should choose the best search strategy in order to maximize efficiency (Stephens and Krebs 1986, Zollner and Lima 1999). The mechanics of movement behavior through an ecological landscape can provide a record of how an animal searches for, interacts with, and uses heterogeneous resources (Turchin 1991, With 1994a). Different movement patterns, or strategies, should be employed in response to different types of landscapes. For example, if resources are aggregated on a landscape, then the best search strategy would be to remain in a patch and continue searching (i.e., area-restricted search). If resources are uniformly distributed on a landscape, dispersal (using ranging movements) immediately after exploiting a resource may be the optimal strategy. Physiological factors such as hunger, sex, age, and mating status may influence searching behavior, introducing both intra-individual and inter-individual variation to the response to resources. Results of work by McIntyre and Wiens (1999b) indicate that food deprivation results in movement pathways quite different than those of satiated beetles. A hungry individual, or one looking for mating opportunities, may move faster and farther than one that is satiated or is not in search of a mate (Bell 1991). Movement patterns can provide a record of the interaction of an organism with the spatial structure of its environment (With 1994a). By studying the movement path of an animal in response to habitat heterogeneity, we may gain insights concerning how it perceives resources on the landscape and may better understand its search strategy.

Edges, corridors, and barriers are landscape boundaries that influence animal movement. John Wiens (1992) has stated very succinctly that, “Patches, boundaries, and heterogeneity, are inextricably linked: Boundaries define patches, and patchiness is what produces heterogeneity”. When one looks at effects of landscape heterogeneity on movement, the importance of boundaries cannot be dismissed. Movement from or into habitat patches depends on probabilities of crossing boundaries between habitat types. There has been considerable interest in how patch size, shape, and boundaries affect plant and animal populations (Stamps et al. 1987, Laurance et al. 2001, Collinge and Palmer 2002) and how these patch characteristics influence animal movement (Hanson and di Castri 1992, Ims 1995, Wiens 1997, Holmquist 1998). More research is needed in this area to understand how response to patch size, shape, and edges influence patch quality and population and community dynamics.

It has been suggested that landscape pattern may not coincide with landscape function (McIntyre and Wiens 2000). Landscape function is determined by the actual use of the habitat for food, shelter, or reproduction and not just by the presence of individuals. The incongruity between pattern and function may be caused by variation in movement behavior among species and individuals. Ability to predict landscape function could be greatly enhanced by both theoretical and empirical studies that examine variations in search behavior and habitat patch selection (Lima and Zollner 1996, McIntyre and Wiens 2000).

Experimental landscape systems

Experimental model landscape systems, small-scale systems on a "microlandscape" level (Wiens et al. 1993), have be used to examine the movement response of insects to heterogeneity and to assess species perception of connectivity of resources. Neutral landscape models are well-suited for the study of the effect of landscape structure on animal movement (With et al. 1999) because they provide a null model of landscape pattern that is produced in the absence of ecological processes (Caswell 1976). They have become valuable tools for the investigation of the effects of landscape structure on ecological processes (Andren 1994, Schumaker 1996, Wiens et al. 1997, With et al. 1997) because they are more easily manipulated, controlled, and replicated than studies conducted at broader scales and/or on naturally generated landscapes.

Results of studies in experimental landscapes have provided new insights into how landscape structure may influence insect movement. Landscape heterogeneity, connectivity, and scales of patchiness have been shown to influence movement in experimental studies on *Eleodes* beetles (Wiens and Milne 1989, Crist 1992, Johnson and Milne 1992, Wiens et al. 1997).

Nonlinear effects of landscape structure on movement have been demonstrated in several experimental studies with beetles (*Eleodes spp.*) and grasshoppers (*Xanthippus* and *Psoloessa spp.*) (With 1994a, With and Crist 1995, Wiens et al. 1997), suggesting that population distributions of animals may not quantitatively coincide with the spatial arrangement of habitat on the landscape. A more broad-scale field study, using an experimental model landscape system, demonstrated that habitat fragmentation and critical thresholds in lacunarity (a measure of average gap size) disrupted the ability of coccinelid predators to aggregate in response to aphid prey (With et al. 2002). Work with *Eleodes* beetles by McIntyre and Wiens (1999a) on experimental landscapes found that movements were influenced both by the presence and the grain of habitat heterogeneity. A specialist goldenrod beetle, *Trirhabda borealis*, moved infrequently and slowly in habitat compared to non-habitat where beetles moved more frequently and faster with sustained directionality (Goodwin and Fahrig 2002).

Studies on various insect species have documented variation in movement behavior in response to habitat versus non-habitat areas in naturally occurring landscape mosaics. The net displacement rate of two damselfly species, *Calopteryx maculata* and *C. aequabilis* from stream habitat decreased in areas with a higher amount of non-habitat pasture compared to landscapes composed of forest and stream habitats (Jonsen and Taylor 2000), indicating that the structure of the broader landscape influenced the ability of these damselflies to travel between streams. The boundary between prairie cordgrass habitat and a non-habitat mudflat area (Haynes and Cronin 2006) was relatively impermeable to planthoppers resulting in aggregation along the interior patch edge. Movements of butterflies inside and outside of habitat patches were quantitatively different; with movement in matrix much straighter than in habitat (Schtickzelle et al. 2007) suggesting travel costs in the matrix may be high and, thus, negatively influence dispersal between habitat patches. Insects have been very amenable for empirical studies of movement on experimental landscapes and the model system approach has provided important insights into the mechanics of animal movement and how these mechanisms may influence population processes.

Objectives

This study examines movement behavior of *T. castaneum* with the aim to elucidate mechanisms underlying movement patterns in patchy landscapes. The purpose of this work is to answer three main questions concerning how various aspects of landscape structure impact movement behavior of red flour beetle:

- How does landscape structure (patch size and abundance of habitat) influence female red flour beetle movement behavior?
- What information is available to female red flour beetle during movement in a landscape? (i.e., do red flour beetles perceive patch structure and evaluate patches in a landscape before physical encounter?)
- How does habitat pattern influence landscape utilization by female red flour beetle for movement and oviposition? (i.e., does the functional use of the landscapes depend on habitat pattern and does it change with different activities?)

The overall hypothesis is that red flour beetle will exhibit quantitatively different movement responses to variation in landscape characteristics, such as scale of patchiness, abundance, and habitat pattern, and to variation in patch quality. Analyses of these responses will then allow identification of behavioral mechanisms important for determining spatial distribution and population structure of red flour beetles. The second chapter focuses on movement behavior in response to landscapes differing in abundance and pattern of habitat in experimental model landscapes. In chapter three, I experimentally examine perceptual range and response to variation in patch boundaries and quality. Chapter four includes experiments on how landscape pattern influences spatial distribution of oviposition sites and consideration as to how this distribution may impact survival. It is my hope that this work will elucidate movement behavior and identify mechanisms used by red flour beetles in response to landscape structure, and that this information will have implications in the areas of behavioral and landscape ecology and applications in stored-product insect ecology.

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CHAPTER 2- Fine-Scale Movement Behavior of Red Flour Beetle (*Tribolium castaneum*): Influence of Landscape Structure on Search Strategies

Abstract

Landscape structure can influence the fine-scale movement behavior of dispersing animals that ultimately may influence ecological patterns and processes at broader scales. The hypothesis that changes in landscape structure generate changes in movement behavior of female red flour beetles (*Tribolium castaneum*) was investigated by observing searching behavior in experimental flour landscapes. Landscape structure was varied by manipulating habitat abundance (0, 10, 30, and 100%) and grain size of patches (fine-2 x 2 cm, intermediate-5 x 5 cm, and coarse-10 x 10 cm) in 50 x 50 cm landscapes. Lacunarity (index describing the variability of gap sizes among locations) analysis indicated an abrupt non-linear change in space use between the coarse-grained and both intermediate- and fine-grained landscapes. Movement pathway metrics indicated that beetles used a similar proportion of all landscape types, but moved more slowly and tortuously (with many turns), and remained longer in both the overall landscape and individual patches, in fine-grained landscapes compared to coarse-grained landscapes. Pathway metrics calculated for intermediate-grained landscapes had values intermediate between the fine and coarse-grained landscapes. Differences in behavioral responses to edges and inter-patch distances may be responsible for observed differences in landscape viscosity, or the degree of resistance to movement.

Introduction

Fine-scale search strategies of dispersing animals may affect ecological patterns and processes at broader scales, ultimately influencing population dynamics (Wiens et al. 1993, Russell et al. 2003, Heinz et al. 2006). For species that depend on specific resources, searching behavior coupled with perceptual range is a basic determinant of searching success (Heinz and Strand 2006) and the degree to which resources may be connected by movement (Taylor et al. 1993, Wiens et al. 1997, Moilanen and Hanski 2001). Connectivity has been defined as the degree that the landscape impedes or facilitates the movement of organisms among patches (Taylor et al. 1993) and functional connectivity relates to how movement behavior and perceptual range of individuals interacts with landscape structure (With and Crist 1995, Schooley and Wiens 2003). Theoretical and empirical ecological research has emphasized the need for understanding how animals perceive and respond to spatial heterogeneity (Ives 1995, Zollner and Lima 1997, With et al. 2002) and the importance of integrating both behavioral and landscape approaches when studying searching behavior (Lima and Zollner 1996, Morales and Ellner 2002, Schooley and Wiens 2003, Heinz et al. 2006). In recognition that population dynamics may be related to behavioral decisions of individuals in response to landscape structure, there has been much recent emphasis on integration of movement behavior into individual dispersal models which simulate dispersal on real and virtual landscapes (Conradt et al. 2003, Russell et al. 2003, Zollner and Lima 2005). To generate accurate parameters and validate dispersal models developed for landscape conservation planning or control of invasive or pest species, more empirical research on searching behavior of focal organisms is needed.

Integration of movement behavior into models of dispersal is based on the premise that population viability is dependent on the dispersal success of organisms, and that this success may depend on an individual's ability to change search strategy depending on the abundance and configuration of habitat (Zollner and Lima 2005, Heinz and Strand 2006). The ability of insects to employ different search strategies in relation to landscape structure has been an active area of research in empirical studies (Bond 1980, Stamps et al. 1987, Bell 1991, Goodwin and Fahrig 2002, Olden et al. 2004, Conradt and Roper 2006). Understanding how shifts in dispersal strategies may impact the distribution and abundance of species requires knowing how individual movement behavior changes in response to changes in habitat abundance and pattern. For example, landscape structure has been shown to impact movement behavior and search success (With and Crist 1995, With and King 1999, With et al. 2002).

To address questions concerning how dispersers may change fine-scale search strategy (or movement rules) in response to landscape structure, I chose to examine the movement behavior of the red flour beetle (*Tribolium castaneum*) on experimental landscapes. Red flour beetles are well-suited for movement studies because they usually disperse by walking, although they are capable of flight. The genus *Tribolium* has a long history as an experimental model in physiology, evolution, genetics, and dispersal due to its ease of culture in the laboratory and its status as a major pest species (Naylor 1961, Dawson 1976, Ritte and Lavie 1977, Costantino and Desharnais 1991, Wade and Goodnight 1991). The focus of earlier studies of dispersal was on movement in bulk grain (Surtees 1964, Hagstrum and Leach 1972) and emigration from food patches (Ziegler 1976) where movement was constrained by the experimental design. There is little information about the influence of patchy resources on movement in landscapes in which beetles may move freely among several patches. More recent studies have begun to examine red flour beetle movement from a landscape perspective, studying movement in terms of behavioral responses to the spatial pattern of resource patches (Campbell and Hagstrum 2002, Campbell and Runnion 2003) movement in response to different substrates and boundaries (Morales and Ellner 2002) and the genetics of dispersal strategies (Korona 1991).

In the current study I created landscapes that differed in structure by varying abundance and the grain size of habitat (flour) and recorded movement pathways of searching beetles on these landscapes. My hypothesis is that differences in landscape structure created by changes in abundance and grain size would generate changes in the search strategies of red flour beetles. Depending on the perceptual range (the minimum distance from which a resource may be detected) and the configuration of habitat, a threshold in the functional, or perceptual grain of the landscape may impact movement behavior of beetles. Using this experimental landscape system, I asked the following questions: How does abundance and grain size of habitat influence beetle movement? Do red flour beetles change dispersal strategy when confronted with different landscape patterns? If dispersal strategy changes, what are the mechanisms involved? To test my hypothesis, I evaluated how a variety of metrics of beetle movement behavior changed with landscape structure.

One useful metric for comparing the spatial pattern of habitat and beetle movement pathways is the lacunarity index (Mandelbrot 1983, Plotnick et al. 1993, With and King 1999, McIntyre and Wiens 2000) that can be calculated for both landscape and movement patterns over multiple scales. The term lacunarity describes landscape texture, or the variability of gap sizes. Therefore, this metric is especially useful for understanding how gaps structure or conversely how habitat contagion or dispersion can influence movement behavior. The lacunarity index provides a measure of how landscape pattern influences the space use of beetles on a landscape.

Methods

Insects

Female beetles used in my study were taken from a lab colony founded with individuals collected from a flour mill ~ 22 months before the experiment was conducted. Sub-colonies were maintained in a wheat/brewers yeast mixture (95:5) at 26 ± 2° C and stored in an environmental chamber at 75 ± 5 % RH. Sub-cultures were maintained by placing ~ 50 beetles (mixed sex) in pint jars containing 0.25 liter of wheat/brewers yeast mixture. Age of the experimental beetles was standardized to 3 weeks ± 4 days by sub-culturing every 3 or 4 days and then removing the initial adults after they had been in the new culture for 3 or 4 days. Sex of beetles was determined by using a microscope to determine the presence or absence of the setaceous patch present on the first femur of males (Good 1936). For the experiment, a group of female beetles (36) were removed from sub-colony jars one day prior to use in the experiment and held in a container with food under the environmental conditions described above. This study was conducted in a walk-in environmental chamber (26 ± 2° C; 75 ± 5 % RH).

Experimental design and landscape creation

I created experimental landscapes with three grain size treatments (2 x 2, 5 x 5, and 10 x 10 cm) within two levels of habitat abundance (10 and 30%). Holding the extent of the landscape constant (50 x 50 cm) while varying grain size produced landscapes with varying degrees of habitat aggregation and heterogeneity of gap sizes (inter-patch distances) (Fig. 1). Previous work with lady beetles, indicated a threshold response to landscape structure with respect to lacunarity when habitat abundance fell below 20% (With et al. 2002). Wiens (1997) also documented threshold effects in movement parameters of tenebrionid beetles when habitat abundance was between 0% and 20%. I chose habitat abundance treatments that bracketed this critical 20% abundance level and that would most likely result in interesting and comparable differences in movement. I also included two control treatments; a 100% and a 0% flour landscape. I chose the landscape extent based on the largest size that was tractable for landscape creation, adequate replication, and accurate visual observation of beetle movement.

Experimental landscape patterns were generated using the freely available RULE software program (Gardner 1999) by creating four different random maps for each abundance x grain size combination so that each treatment was blocked four times. RULE outputs binary maps on a regular grid of cells. On my landscapes, habitat cells were comprised of unbleached, white flour and matrix cells that did not contain flour. The binary map produced by the computer program was copied onto a piece of heavy cardstock marked with a grid of cells corresponding to the appropriate grain size treatment. Cells designated as habitat were then cut out creating a template for each landscape treatment. I placed the templates on a new sheet of white paper, marked with a grid, within a 58 x 58 cm arena and sifted a fine layer of flour over the template. Flour was applied at a depth shallow enough (< 1 mm) to allow observation of the grids and beetles in habitat cells. The arena was surrounded on all four sides by meter-high white foam-core walls designed to reduce air movement and visual cues from the surroundings. Three light fixtures with 40-watt incandescent bulbs were suspended ~ 1.75 m over the arena providing a relatively low light level ranging from 260 - 290 lux for all replicates.

I released one female beetle into the matrix after a 3-min acclimation period under an inverted 14.8-ml glass vial placed in the center of the landscape. Landscapes were chosen so that beetles would only be released into matrix and not into habitat cells. After release, I recorded which cells on the landscape grid the beetle occupied at 2-sec intervals (i.e., each 2-sec interval was treated as one time step of a beetle movement pathway) for a maximum of 3 min or until she crossed the boundary of the landscape. The 6 abundance x grain size treatments and 2 controls were randomized and replicated 3 times (each with an individual beetle) per day. One of the 4 maps corresponding to each treatment was used for all replicates within each day. The experiment was repeated sequentially over 8 days with the 4 maps being used twice over the course of the experiment. This design resulted in each treatment being replicated 24 times.

Relative effects of landscape structure on beetle movement

I characterized the spatial distribution of both habitat cells and beetle movement pathways by calculating lacunarity indices for the six abundance x grain size habitat patterns and for beetle movement pathways on each landscape. To calculate lacunarity, a box (window) representing a specific scale of measurement (e.g., 10 x 10 cm) is systematically moved across the rows and columns of a regular grid on which is mapped binary (presence–absence, 0,1) data of a spatial pattern. Gaps in the pattern are measured using a moving window algorithm that calculates mean and variance values of the scores of 0's within each box, converting them into an index that is a ratio of the calculated mean and variance values. For landscape pattern, a high lacunarity value indicates large and more variable gaps between more aggregated habitat cells, whereas a low lacunarity value results when habitat cells are more widely dispersed and gap sizes are smaller and more uniform. For movement pathways, a high lacunarity value indicates that cells through which beetles have moved are restricted to a localized area (aggregated) and there are large or irregular gaps in the pattern, while a low value occurs when cells through which beetles have moved are scattered over a wider area of the landscape and gaps are more regularly spaced.

Due to the design of the experiment, the largest cell size in my experiment constrained the grain of the analysis so it was not possible to use lacunarity index values at scales finer than the size of the largest habitat cell. Lacunarity index values can be calculated over a range of measurement scales (box sizes) based on grain size. To ensure that lacunarity measures were comparable I used three lacunarity values per landscape calculated at equivalent scales of measurement across all landscapes; the first was at the scale of the largest grain size, 10 x 10 cm (scale 1), the second was at 20 x 20 cm (scale 2), and the third was at 30 x 30 cm (scale 3). To provide additional measures for relative comparisons of landscape structure and movement behavior, I quantified the following metrics for each landscape: total number of habitat patches (all adjacent habitat cells, including diagonals), total edges (sum of lengths of all habitat edge segments), nearest neighbor distance (distance to nearest habitat cell), and largest patch index (percent of total area composed of largest habitat patch).

Beetle movement patterns were quantified using a variety of measures in addition to lacunarity. I quantified immigration into all cells, both matrix and habitat, as a measure of landscapes connectivity and immigration into just the habitat cells as a measure of habitat connectivity. Since the area of large and medium cells were greater than small cells by factors of 5 and 2.5, respectively, I multiplied cell counts for large and medium cells by these factors in order to standardize the area of habitat cells so that metrics for immigration and time steps within habitat cells would not be biased due to size differences among habitat cells. I calculated three metrics for each beetle movement pathway: (1) the mean step length, *i.e.,* distance moved during each 2 s time step; (2) the displacement ratio, *i.e.,* computed as the net displacement (a straight-line measure of the pathway) divided by total path length (this ratio is a measure of pathway complexity standardized for different observation times [completely linear pathway = 1]); and (3) displacement rate, *i.e.,* the net displacement divided by time step (2-second interval). The amount of time spent within landscapes and in individual habitat and matrix cells can also indicate how beetles respond to landscape structure. Therefore, I calculated the number of time steps in the overall landscape, in habitat cells and in matrix cells; as well as the number of time steps within individual habitat and matrix cells. I recorded the time that beetles remained in the landscape as a measure of how habitat structure influenced retention time in the landscape.

Data analysis

I used analysis of variance (ANOVA) (mixed-model procedure, (SAS Institute, Inc. 2002) to test for differences in metrics describing the landscape using the treatment combination of habitat abundance and habitat grain size modeled as the main effect and maps and blocks modeled as random effects. To compare lacunarity (distribution) of beetle movement versus that of the landscape I used ANOVA (mixed-model procedure) with scale, abundance, and grain size modeled as main effects and maps and blocks as random effects. Lacunarity values were log-transformed before analysis to normalize data. To test the effects of grain size and abundance on movement behavior I performed an ANOVA (mixed-model procedure) on beetle movement pathway metrics with habitat grain and abundance modeled as main effects and maps and blocks as random effects. Effects of habitat abundance on pathway metrics were analyzed separately from grain size effects because pathway data for controls were recorded only at one grain size (2 x 2 cm) and calculation of several metrics (e.g., time steps in habitat) were not possible for habitat abundance controls. The Residual Maximum Likelihood (REML) method was used to estimate the variance components of the mixed models. Tukey HSD was used for separation of means. Time that beetles remained on the landscape was analyzed using survival analysis (Proc Lifetest; SAS Institute, Inc., 2002) for censored data with a Bonferroni correction for multiple comparisons.

Results

Landscape structure

Landscape metrics

Landscape metrics characterizing the various landscapes showed differences due to the combined effects of habitat abundance and grain size. Analysis of variance revealed significant differences among treatments for all landscape metrics: number of patches (F = 305.02; df = 5,127; $p < 0.0001$); largest patch index (F = 210.62; df = 5, 127; $p < 0.0001$); total edges (4482.25; df = 5,127; $p < 0.0001$); and nearest neighbor distance (F = 12.28; df = 5,127; $p < 0.0001$) (Figure 3). Changes in landscape structure due to habitat abundance and grain size did not impact all patch metrics in the same manner. For example, the effect of habitat abundance on landscape structure is most apparent for the largest patch index and total edges within each grain size treatment. There is a dramatic increase in number of habitat patches in fine-grained landscapes compared to the intermediate and coarse-grained landscapes. As grain decreases and abundance increases landscapes have significantly more habitat patches and, thus, more edges, while nearest neighbor distance decreases. The greatest difference in total edges occurs between the 10% coarse-grained and the 30% fine-grained landscapes.

Lacunarity

The wide range of lacunarity values associated with landscapes indicated differences in distributions of habitat cells and heterogeneity in gap sizes (Figure 2A). A full-factorial analysis of both landscape pattern and movement path lacunarity revealed significant differences between lacunarity of movement paths and that of landscape (F = 40.65; df = 1,16; $p < 0.0001$) (Figure 2). To examine sources of variation, lacunarity of landscape pattern and beetle movement paths were analyzed separately. The separate analysis of landscape lacunarity again showed significant differences between abundance levels (F = 64.70; df = 1,4; p = 0.0013); among scales (F = 123.56; df = 2,4; p = 0.0003); and among grain sizes (F = 199.45; df = 2,4; p < 0.0001) (Figure 2). Landscape pattern lacunarity values were higher and had more variation among treatments, especially among both coarse-grained and the intermediate-grained 10% habitat landscapes. Lacunarity values were lower and were less variable for the fine-grained and the 30% intermediate-grained landscapes. Higher lacunarity values in one group of landscapes (coarse and 10% intermediate-grained) indicate that habitat cells are more aggregated and inter-patch distances are larger and more variable in size than in the other landscape group (fine and 30% intermediate-grained) where habitat cells are more evenly dispersed and inter-patch distances are smaller and more regularly spaced (Figure 1).

Beetle movement pattern

Space use at the landscape scale

Lacunarity analysis revealed that grain size (F = 96.56; df = 2,4; p = 0.0004) significantly impacted the space use of beetles in the different landscapes while habitat abundance did not (F = 0.01; df = 1,4; p = 0.9123) (Figure 2B). Because there was no effect of habitat abundance, nor interaction between abundance and grain size, data were pooled and a reduced model examined the influence of grain size and scale of measurement on lacunarity of beetle movement pathways. As expected, lacunarity significantly decreased in all landscapes because, as box size increases, variation among locations is reduced (F = 1703.64; df = 2,9; p < 0.0001) (Figure 2B). There were no significant differences between lacunarity of beetle movement pathways in landscapes with 0% habitat and those with 100% (F = 0.00; df = 1,2; p = 0.9931) (Figure 2B). There was an abrupt non-linear response in beetles' use of space between the coarse-grained landscapes and the fine-grained and intermediate landscapes at the finest measurement scale (scale 1, 10 x 10 cm) (F = 86.63; df = 2,9; p < 0.0001) (Figure 4A). In coarse-grained landscapes, lacunarity values were higher, thus there were large and irregular gaps among locations of movement, indicating that beetle movement was more linear. This pattern held even at the two larger scales of measurement (scales 2 & 3) although the change between the coarse-grained and the other landscapes is less abrupt (Fig 4B&C).

Movement path response

Connectivity of overall landscape cells (number of landscape cells in which beetles moved) was similar among all grain size treatments (F = 2.38; df = 2,126; p = 0.096) (Table 1), as well as between abundance levels (F = 0.01; df = 1,126; p = 0.943) (Table 2). Connectivity of habitat cells (number of habitat cells into which beetles moved), while significantly higher in landscapes with 30% than in 10% habitat (F = 69.00; df = 1,126; p < 0.0001) (Table 2), followed the proportional difference of habitat abundance between the two treatments. Unexpectedly, grain size was not a significant factor in connectivity of habitat cells (F = 0.04; df = 2,126; p = 0.961) (Table 1).

Beetles spent 36% more time in fine and 23% more time in intermediate-grained landscapes than in coarse-grained landscapes (F = 9.36; df = 2,126; p < 0.0001) (Table 1). Beetles spent less than half the amount of time in landscapes with no habitat (0%) than in landscapes with habitat, and significantly less than those with 30% or higher (F = 8.75; df = 3,169; p < 0.0001) (Table 2). Beetles moved in a significantly more tortuous manner in fine and intermediate-grained landscapes than in coarse-grained landscapes (F = 7.22; df = 1,126; p = 0.001) (Table 2). This result agrees with that of the lacunarity analysis, showing that the movement pattern was more linear in the coarse-grained landscapes. Habitat abundance did not impact the tortuosity of movement pathways in landscapes (F = 0.70; df = 1,169; p = 0.554) (Table 2). Beetles' displacement rate was 2 times faster in coarse-grained and 1.2 times faster in intermediate-grained than on small-grained landscapes (F = 4.61; df = 2,126; p = 0.012). There were no significant differences in displacement rate between fine and intermediate-grained landscapes ($p = 0.216$) (Table 1). Beetles' displacement rate in landscapes where there was no habitat present (0%) was 1.7 times higher than in landscapes with 10% habitat, 2.8 times higher than in landscapes with 30% habitat, and 14 times faster than on landscapes with 100% habitat ($F = 12.60$; $df = 3,169$; $p < 0.0001$) (Table 2). Displacement rate was 1.6 times faster on landscapes with 10% habitat compared to those with 30% ($p = 0.028$) (Table 2).

Grain size had no impact on the distance moved (i.e., number of cells visited) during a time step ($F = 1.12$; $df = 2,126$; $p = 0.330$). Distance beetles moved during a time step was 1.6 times greater in landscapes with no habitat present (0%) compared to those with 10% and was ~ 1.3 times greater in landscapes with 10% habitat compared to those with 30 % and 100% ($F = 14.11$; $df = 3,169$; $p < 0.0001$) (Table 2). Beetles spent roughly twice as much time in habitat in landscapes with 30% habitat abundance relative to those with 10% ($F = 23.48$; $df = 1,126$; $p < 0.0001$) (Table 2). This difference represented a 70% increase (18.53 steps) in addition to the 20% (4.13 steps) increase that was expected due to the increase in habitat abundance. The reciprocal was also true, with time spent in the matrix also being influenced by the amount of habitat, with beetles spending more time in matrix in landscapes with 10% relative to 30% habitat ($F = 5.99$; $df = 1,126$; $p = 0.016$) (Table 2). Interestingly, grain size played no role in the amount of time spent in habitat ($F = 1.40$; $df = 2,126$; $p = 0.250$) (Table 1), but did have a significant influence on time in the matrix ($F = 8.71$; $df = 2,126$; $p = 0.0003$). Beetles spent similar amounts of time in the matrix in fine and intermediate-grained landscapes, but at least 1.5 times longer than in coarse-grained landscapes ($p = 0.406$) (Table 1).

Grain size influenced time spent in individual habitat cells, with beetles spending 58% more time in individual habitat cells in fine-grained relative to coarse-grained landscapes ($F = 3.19$; $df = 2,126$; $p = 0.034$) (Table 1). Time spent in individual habitat cells on intermediate-grained landscapes was not significantly different from that in either fine or coarse-grained landscapes (intermediate vs. fine, p = 0.397; intermediate vs. coarse, p = 0.450. Beetles spent 33% more time in individual matrix cells in fine-grained compared to coarse-grained landscapes (F = 4.41 ; df = 2,126 ; p = 0.014) and the amount of time beetles spent in matrix cells of intermediate-grained landscapes was intermediate between the two (Table 1). Habitat abundance did not have a significant effect on amount of time spent per individual habitat cell (F = 0.13; df = 1,126; p = 0.724) nor individual matrix cells (F = 0.95; df = 1,126; p = 0.331) (Table 2). For all pathway metrics there were no significant grain size and abundance interactions.

Landscape retention time

The time that beetles remained in landscapes varied significantly with landscape structure (Figure 5, Table 3). Beetles remained longer in the 30% fine-grained landscapes relative to the other landscapes. In fine and intermediate-grained landscapes with 30% habitat, beetles remained much longer than in landscapes with 100% habitat. Retention time in landscapes with no habitat (0% control) was significantly lower than for all other landscapes with habitat present with the exception that the 10% coarse-grained landscape had a similar low retention time (Table 3).

Discussion

Red flour beetles shift search strategy in response to changes in landscape structure. This shift occurs as a result of behavioral mechanisms employed in response to the scale of the habitat pattern. At the extent of landscapes in this study, beetles accessed a similar proportion of habitat, but fine-scale responses indicated that beetles were searching these landscapes very differently depending on patch and gap structure of the landscape. Differences in movement behavior between coarse-grained and fine-grained landscapes, supported by both movement lacunarity and pathway metric analyses, show that beetles employed two distinct behavioral strategies, occurring in response to the two extremes of grain size in this study. As the grain of habitat increased, and consequently gap size, beetles appeared unable to perceive habitat that was not in close proximity; thus, engaged in a generalized search strategy, employing increased velocity and a linear trajectory. On coarse-grained landscapes, movement pathways indicate that beetles may have perceived themselves as "out of patch". In fine-grained landscapes, pathways were very tortuous and displacement rate low even though mean step length was similar to the other landscapes. Beetles in these landscapes appeared to have perceived themselves as still in a patch even though part of their pathway was in matrix. These perceptual differences and resultant behavioral modifications may ultimately have population consequences by altering aggregation propensities of individuals (Turchin 1989, With and Crist 1995) and thus habitat colonization patterns. Spending more time inside or in proximity of patches could increase colonization because red flour beetles often return to previously explored habitat patches (Romero 2007). Increased time spent in patches could also increase colonization probabilities because red flour beetles use aggregation pheromones for attraction of conspecifics to previously uncolonized habitat (Boake and Wade 1984).

Results of this work and that of others indicate that variation in landscape resistance to movement may be a useful indicator of an organism's perception of being in acceptable or less acceptable habitat. With (1994) examined movement of large and small species of grasshoppers in relation to landscape heterogeneity and found significant differences in rate of movement and pathway tortuosity among the species. She proposed that differences in perceptual resolution of the scale of patch structure affected movement behavior and this impacted relative permeabilities of the landscapes. The fine-grain landscapes in the present study could be described as being relatively resistant to movement while coarse-grain landscapes could be considered relatively more permeable. Intermediate-grained landscapes seem to lie somewhere in between, with some pathway metrics aligning with the fine-grained and some aligning with the coarse-grained landscapes. However, lacunarity index values of intermediate landscapes were similar to the fine-scale landscapes. Differences in resistance to movement with respect to landscape elements considered to be habitat versus non-habitat have also been described in other systems. Goodwin and Fahrig (2002) reported that a specialist goldenrod beetle, *Trirhabda borealis*, moved infrequently and meandered slowly in goldenrod patches compared to cut patches (non-habitat) where beetles moved more frequently with sustained directionality. Haynes and Cronin (2006) reported that planthoppers exhibited greater step lengths, shorter residence times, higher displacement rates, and lower fractal dimension of movement paths (lower tortuosity) in non-habitat mudflat areas. In another study, the net displacement rate of various damselfly species increased in areas with a higher amount of non-habitat pasture compared to landscapes composed of only forest and stream habitat (Jonsen and Taylor 2000). A recent study by Schtickzelle et al. (2007) revealed that movements of butterflies inside and outside of habitat patches were quantitatively different with movements in matrix much straighter than in habitat.

I suggest that edge effects and small inter-patch distances most likely impacted behavioral responses of red flour beetles in fine-grain landscapes. Beetles moving in fine-grained landscapes encountered a high number of edges and did not have far to travel before encountering another edge, thus traveling at a much slower rate and in a more complex manner. For instance, results show that beetles spent disproportionately more time (70% more than expected due to increase in habitat) in overall habitat on landscapes with 30% versus 10% habitat. Survival analysis showed that beetles remained longest in the 30% fine-grained landscape which had the smallest inter-patch distances and the highest number of edges. In contrast, the 10% coarse-grained landscape, with the largest inter-patch distances and the least number of edges, had the lowest retention time of all landscapes, not including the 0% control landscapes where beetles, predictably, left quite quickly. This increased time may be explained by the observation that beetles often moved along edges of habitat cells both inside and outside the cell. Once entering a habitat cell, beetles followed along interior edges of cells rather than transition immediately back out into the matrix. Beetles also spent more time in fine-grained habitat cells versus coarse-grained habitat cells. Beetles appear to be making fewer transitions out of fine-grain habitat cells than out of coarse-grain habitat cells. This behavior could be explained by increased edge encounters in the fine-grain cells that reflect beetles back into interior. Fine-scale response to edges is likely to be responsible, at least in part, for the decreased dispersal rate and increased retention time on fine-grained landscapes. These observations add to and further corroborate previous studies showing that differences in patch viscosity and edge permeabilities may be contingent on the structure of the surrounding landscape. For example, in a study of tenebrionid beetles moving in experimental landscapes of grass and bare ground, Wiens (1997) implied that viscosity of grass patches may not be constant but may vary with the overall coverage of grass. He attributed this difference in viscosity to alteration of behavior at patch edges (e.g., stopping at patch edges) because these effects will accumulate in landscapes with a high edge-to area ratio of habitat patches (e.g., fragmented). In a similar manner, crickets moving among grass patches imbedded in sand used habitat significantly more than expected in 20% patchy landscapes compared to clumped landscapes (With et al. 1999).

Landscapes have been described as cost-benefit surfaces (Wiens 2001) whereby dispersers may incur costs from mortality or loss of fitness as they travel between suitable patches of habitat. Dispersal costs are likely to increase with increasing inter-patch distance and anthropogenic habitat fragmentation, making dispersal success less likely. Zollner and Lima (1999) predicted that movement should be more linear when animals are facing greater risks, such as searching in an inhospitable matrix for suitable habitat, than when moving through a benign matrix. Variation in dispersal success in patchy landscapes may be mitigated if individuals have the ability to respond to habitat structure by changing their search strategy (Roitberg and Mangel 1997). The ability of an animal to modify its searching behavior is related to the information the individual receives concerning the distribution of resources as it is moving on the landscape and the amount of information received is determined by its perceptual range. Recently, Baguette and Van Dyck (2007) proposed that the grain of resource configuration in landscapes is a crucial factor shaping individual movements. The authors suggest that functional grain, or the scale of interaction between organism and the landscape, will depend on whether or not the grain of resource patches matches the spatial scale of the perceptual range. For red flour beetles there certainly seems to exist a critical functional grain size at which interactions with habitat and, thus, search strategy changes. Beetle movement behavior exhibited a dichotomy between the coarse and both the fine and intermediate-grained landscapes, suggesting that the perceptual range of red flour beetles in this study could be less than 12 centimeters, the average nearest neighbor distance between habitat patches of the intermediate and coarse-grained landscapes.

A behavioral-based approach for investigating a species distribution in real landscapes (Lima and Zollner 1996) requires knowledge concerning how perceptual range may influence search strategies in response to landscape structure. This research has demonstrated how detailed examination of movement pathways and measures of lacunarity can be useful in determining functional grain and how a species may modify search strategies in response to functional grain. Spatially explicit, organism-centered studies focusing on behavioral responses to different habitat configurations can serve as an important first step to identify behavioral rules of movement that may ultimately lead to more accurate predictions of space use in landscapes.

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Figures and Tables

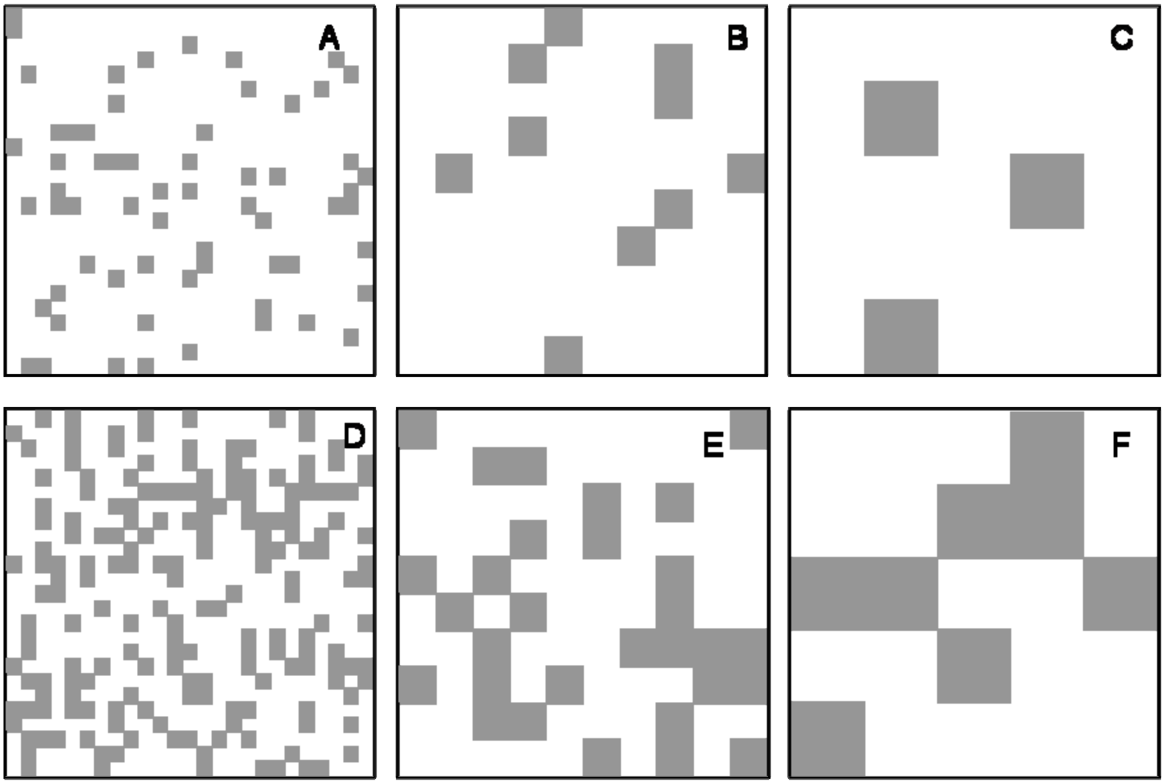

Figure 2-1 Experimental landscapes (each 50 x 50 cm) showing heterogeneity of landscape structure (pattern and size of habitat cells and gaps between habitat cells): (A) 10% fine-grained, (B) 10% intermediate-grained, (C) 10% coarse-grained, (D) 30% fine-grained, (E) 30% intermediate-grained, and (F) 30% coarse-grained.

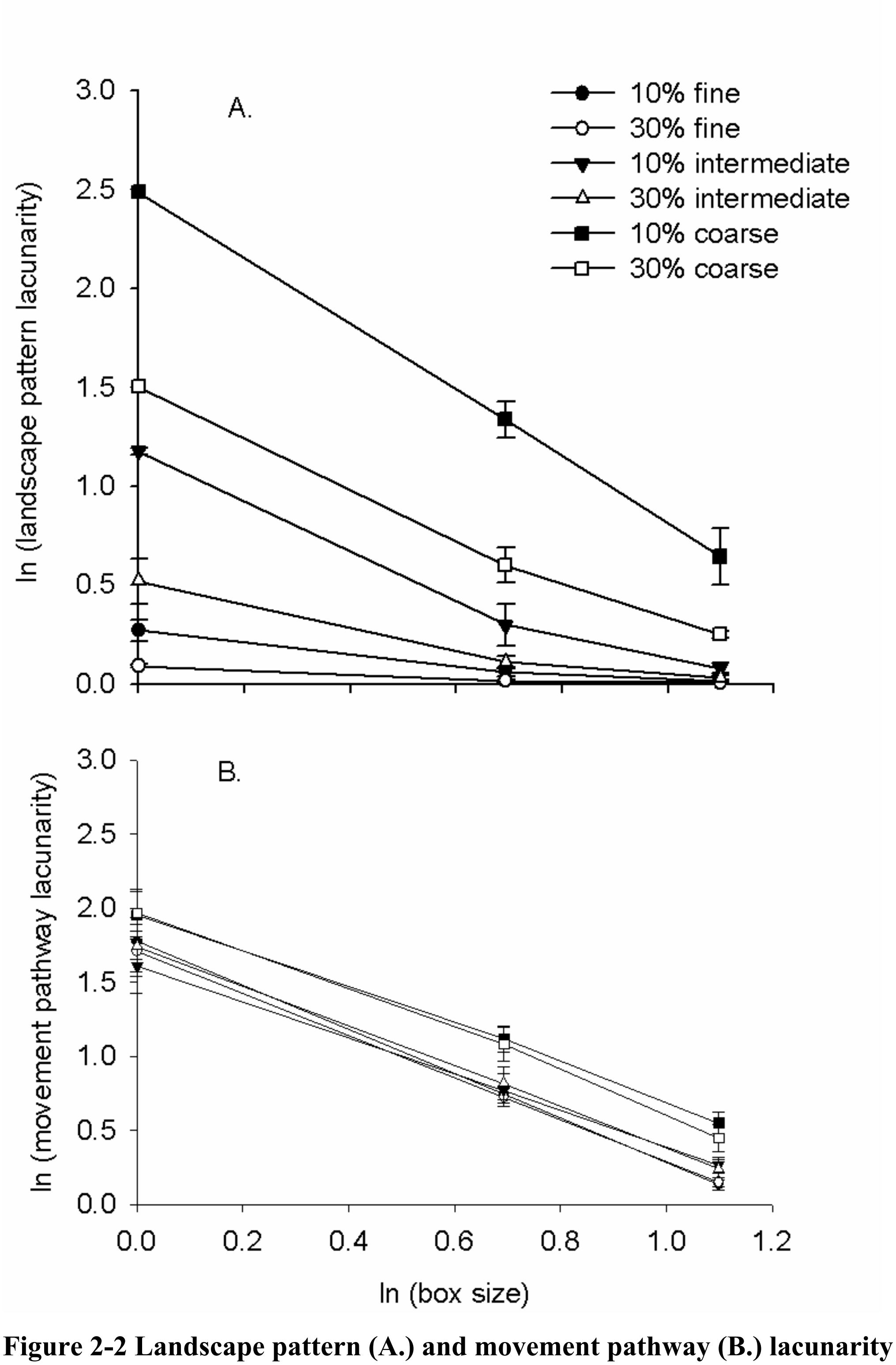

Figure 2-2 Landscape pattern (A.) and movement pathway (B.) lacunarity plotted across three measurement scales (box sizes) showing relationship of habitat abundance and grain size to landscape structure and movement behavior of red flour beetles (*Tribolium castaneum*). Values on axes were log transformed for display purposes.

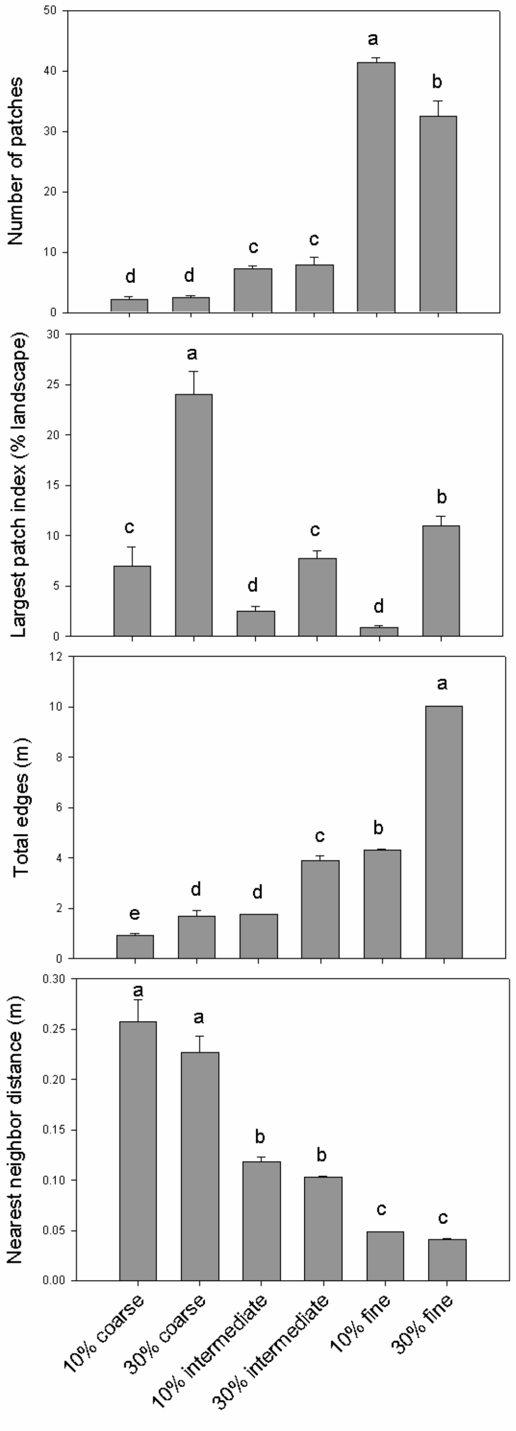

Figure 2-3 Landscape metrics as functions of habitat abundance and grain size. Treatments with the same letter are not significantly different within a plot.

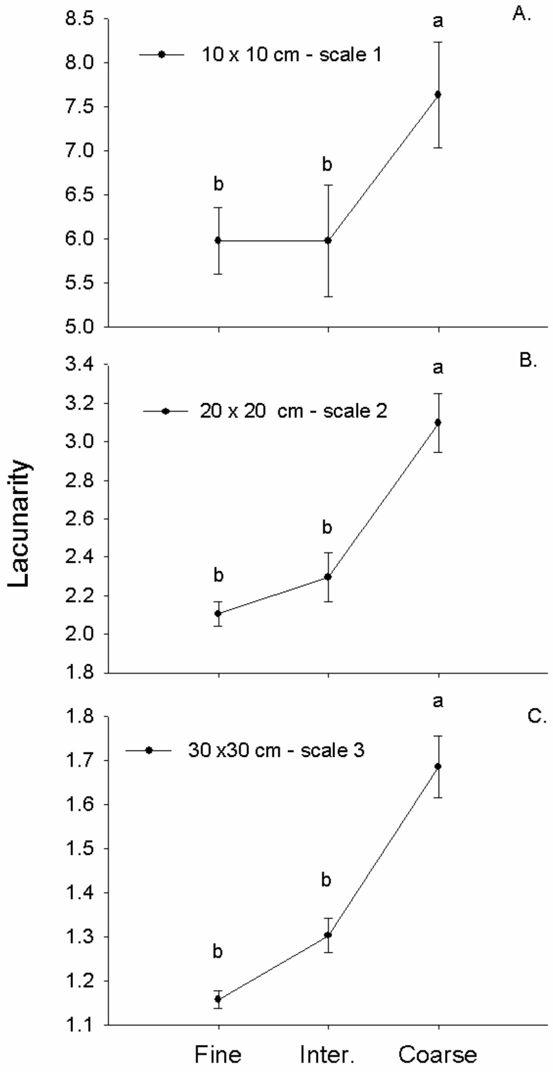

Figure 2-4 Lacunarity of red flour beetle (*T. castaneum*) movement pathways as a function of habitat grain size over three scales of lacunarity (box size) including: A.) scale 1 - 10 x 10 cm, B.) scale 2 – 20 x20 cm, and C.) scale 3 – 30 x 30 cm. Symbols with same letters are not significantly different (ANOVA, mixed procedure, alpha = 0.05).

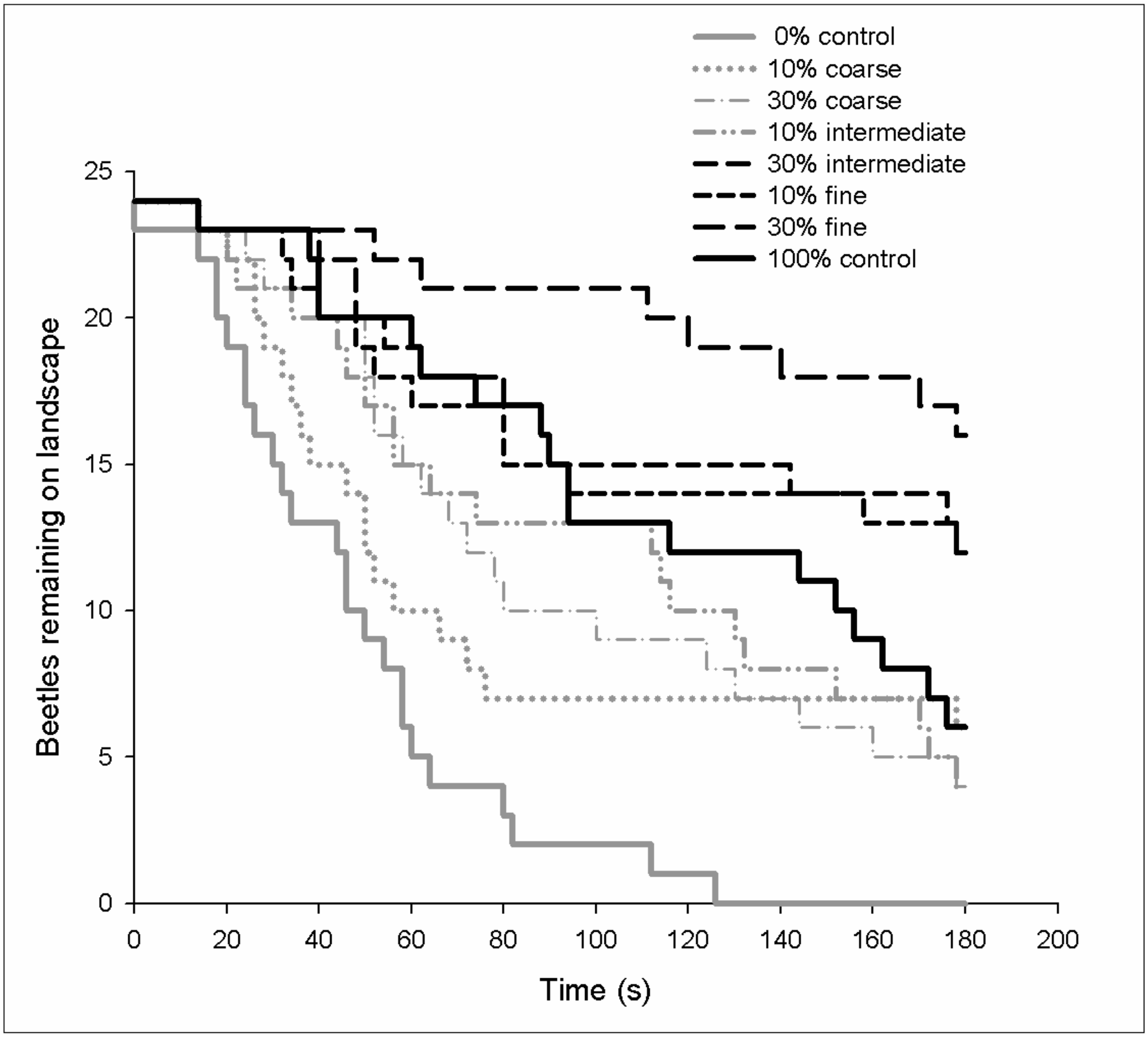

Figure 2-5 Retention time curves for number of beetles (*T. castaneum*) remaining in landscapes as a function of landscape structure. Observations were censored after 180 seconds.

Table 2-1 Effects of grain size on movement behavior of female red flour beetle (*T. castaneum*) at various scales in experimental landscapes. Means ± SE with same letters within rows are not significantly different (ANOVA, mixed procedure, alpha = 0.05).

Scale, Pathway, and behavior measures	Grain size		
	Fine	Intermediate	Coarse
Overall landscape			
Landscape cells connected	28.16 ± 2.47 a	32.37 ± 2.48 a	26.66 ± 2.47 a
Habitat cells connected	6.63 ± 0.71 a	6.66 ± 0.71 a	6.88 ± 0.71 a
Time steps on landscape	71.21 ± 4.23 a	59.44 ± 4.27 a b	45.52 ± 4.23 b
Displacement ratio	0.29 ± 0.04 b	0.36 ± 0.04 b	0.49 ± 0.04 a
Displacement rate	0.004 ± 0.000 b	0.006 ± 0.000 a b	0.008 ± 0.000 a
Between cells			
Mean step length	0.015 ± 0.002 a	0.017± 0.002 a	0.017 ± 0.002 a
Time steps in habitat	35.42 ± 4.96 a	28.49 ± 4.99 a	25.54 ± 4.96 a
Time steps in matrix	35.85 ± 3.05 a	30.72 ± 3.07 a	19.75 ± 3.05 b
Within cell			
Time steps per habitat cell	7.55 ± 1.36 a	5.28 ± 1.37 a b	3.19 ± 1.36 b
Time steps per matrix cell	1.82 ± 0.15 a	1.40 ± 0.15 a b	1.21 ± 0.15 b

Table 2-2 Effects of habitat abundance on movement behavior of female red flour beetle (*T. castaneum*) at various scales in experimental landscapes. Means ± SE with same letters within rows are not significantly different (ANOVA, mixed procedure, alpha = 0.05).

Scale, Pathway, and behavior measures	Habitat Abundance			
	0%	10%	30%	100%
Overall landscape				
Landscape cells connected	21.750 ± 2.867 a	29.17 ± 1.655 a	28.97 ± 1.667 a	28.167 ± 2.867 a
Habitat cells connected	-	3.45 ± 0.59 b	9.99 ± 0.59 a	-
Time steps on landscape	24.750 ± 10.970 b	52.9 ± 3.45 a b	64.51 ± 3.47 a	58.542 ± 10.970 a
Displacement ratio	0.528 ± 0.840 a	0.395 ± 0.037 a	0.367 ± 0.037 a	0.393 ± 0.084 a
Displacement rate	0.014 ± 0.001 a	0.008 ± 0.001 b	0.005 ± 0.001 c	0.006 ± 0.001 b c
Between cells				
Mean step length	0.028 ± 0.003 a	0.018 ± 0.001 b	0.014 ± 0.001c	0.015 ± 0.003 b c
Time steps in habitat	-	20.67 ± 4.09 b	39.20 ± 4.10 a	-
Time steps in matrix	-	32.19 ± 2.46 a	25.35 ± 2.47 b	-
Within cell				
Time steps per habitat cell	-	5.58 ± 1.14 a	5.12 ± 1.15 a	-
Time steps per matrix cell	-	1.39 ± 0.12 a	1.56 ± 0.12 a	-

Table 2-3 Pair-wise comparisons of times until female red flour beetles (*T. castaneum*) leave experimental landscapes. Means ± SE reported are for biased mean leaving time (s). Comparisons of retention curves (Kaplan-Meier method) are significantly different at $p \leq 0.0017$ after Bonferroni correction for multiple comparisons. Significance level between pairs in rows and columns are indicated by p – values in bold type.

Landscape comparisons		10% coarse	10% inter.	10% fine	30% coarse	30% inter.	30% fine	100%
	Mean ± S.E.	P - value						
0%	49.50 ± 6.16	0.024	**0.0001**	**< 0.0001**	**0.0004**	**<0.0001**	**<0.0001**	**<0.0001**
10% coarse	54.42 ± 4.42	_	0.717	0.032	0.672	0.026	**0.0005**	0.319
10% intermediate	104.67 ± 12.22	_	_	0.044	0.824	0.037	**0.0003**	0.588
10% fine	116. 92 ± 11.39	_	_	_	0.035	0.984	0.163	0.123
30% coarse	94.17 ± 10.48	_	_	_	_	0.027	**0.0002**	0.379
30% intermediate	131.22 ± 12.52	_	_	_	_	_	0.160	0.115
30% fine	151.33 ± 8.95	_	_	_	_	_	_	**0.001**
100%	116.08 ± 11.34	_	_	_	_	_	_	_

CHAPTER 3- Behavioral Response of Red Flour Beetle (*Tribolium castaneum*) to Patch Boundaries: Role of Perceptual Range and Permeability

Abstract

Exploitation of resource patches is influenced by both the distance from which animals perceive suitable habitat (perceptual range) and the permeability of patch boundaries and these interactions can play a key role in determining dispersal success and ultimately population distribution. This study examined perceptual range in female red flour beetle (*Tribolium castaneum*) by manipulating distance, hunger level, and airflow and measuring the number of individuals successfully locating a flour patch (i.e., goal) in a wind tunnel. Results of perceptual range experiments measuring beetle attraction to patches suggest that beetles do not detect patches until in close proximity to the patch edge, and that hunger level of beetles and presence of air flow over the patch did not significantly increase patch detection. Patch permeability was investigated by recording movement pathways near boundaries, boundary transitions, and retention time in response to patches with different amounts of resource and shelter. Beetles crossed boundaries of sheltered patches (covered) more quickly compared to high- and low-resource treatments without cover, suggesting that shelter may be an important factor in determining permeability. At initial contact, high-resource patches were significantly less permeable to beetles than low-resource patches, but after additional encounters beetles more readily entered high-resource patches compared to low-resource patches. Results suggest that shelter and permeability of patch boundaries may play an important role in controlling movement patterns of red flour beetles.

Introduction

The spatial distribution of animals inhabiting landscapes where habitat is patchily distributed is largely determined by the distance from which they can detect patches (perceptual range) and their movement behavior as they move from one patch to another (Bell 1991, Ims 1995, Zollner and Lima 1997, Turchin 1998). Population and metapopulation persistence is determined by the connectivity of patches and landscapes (Taylor et al. 1993, Tischendorf and Fahrig 2000). Connectivity has been defined as the degree that the landscape impedes or facilitates the movement of organisms among patches (Taylor et al. 1993) and functional connectivity relates to how movement behavior and perceptual range of individuals interacts with landscape structure (With and Crist 1995, Schooley and Wiens 2003) .

For animals searching for suitable habitat in patchy landscapes, the extent of their perceptual range has a significant effect on how they perceive the landscape structure and, as a result, the time they may spend and the manner in which they search for habitat. For example, an animal with a small perceptual range may need to spend more time searching and employ a more complex movement pattern than an animal with a large perceptual range that can perceive a patch from a distance and move directly to a new patch. Perceptual range may vary with both environmental and physiological conditions, thus impacting movement behavior and search success (Schooley and Wiens 2003) .

Functional connectivity may also be impacted by how animals interact with landscape boundaries such as such as edges, corridors, and barriers (Hanson and di Castri 1992, Lidicker and Koenig 1996, Haddad 1999). Corridors and barriers may channel movement influencing positively or negatively the ability of an animal to find suitable habitat. Movement from or into habitat patches depends on probabilities of crossing boundaries between habitat types which may depend on the animal's perception of the edge. Characteristics of both the boundary and the dispersing animal determine permeability, or the probability that a boundary will be crossed (Stamps et al. 1987, Wiens 1992). Patch characteristics that influence boundary permeability include: size, shape, and the contrast between the patch and the surrounding area. Characteristics of dispersers that influence permeability of habitat patches are many and may include: population density, movement patterns, perceptual range, and physiological state. Behavior of individuals at habitat boundaries can impact population distribution in patchy environments by controlling the flux of patch transfers (Wiens 1992). There has been considerable interest in how landscape boundaries affect distributions of plant and animal populations (Hanson and di Castri 1992, Wiens et al. 1993, Tischendorf and Fahrig 2000, Laurance et al. 2001). Despite this interest, knowledge is limited concerning the impact of boundaries due to lack of empirical studies on animal movement, especially those focusing on how an animal behaves when encountering habitat boundaries (but see With 1994a, Holmquist 1998, Haddad 1999, With et al. 1999, Ries and Debinski 2001, Goodwin and Fahrig 2002, Morales 2002).

Current theory recognizes that dispersal is a complex process composed of at least three phases: an initial decision to emigrate, a searching phase in which decisions are made by individuals as they move between habitat patches, and a colonizing phase where dispersers immigrate into new habitat. Most information on behavior at boundaries concern interactions that occur as individuals make decisions to leave the patch or not (Stamps et al. 1987, Ries and Debinski 2001). Few studies have examined what happens after the decision to emigrate has been made, such as how a disperser locates new habitat patches while moving through a landscape and how it responds when encountering the boundary of a new habitat patch. In this study I focus on two important aspects of the last two phases of the dispersal process: detection of new habitat during the searching phase and behavior at the boundary of a new habitat patch at the beginning of the colonizing phase. I have two specific objectives: the first is to investigate the perceptual range of red flour beetle (*Tribolium castaneum*) by determining how they respond to habitat patches prior to encountering the physical patch boundary and the second is to examine how habitat patches with different characteristics (quality) affect boundary permeability.

Red flour beetle is a cosmopolitan, stored-product pest insect that is well-recognized for its ability to find and colonize patchily distributed habitat. There have been very few studies addressing movement behavior of stored-product insects in patchy landscapes even though these insects are frequently found in these kinds of environments. The landscapes in which stored-product insects live are composed of spatially separated food patches linked by movement and, at a given time, only a portion of the patches may be occupied by the insects. Probabilities that insects within these spatially separated patches will be killed or disturbed by pest control measures differ and so successful dispersal from one patch to another drives the ability of stored-product insect populations to persist and challenges the efficacy of control tactics (Campbell and Hagstrum 2002). Understanding red flour beetle movement behavior during the searching and colonizing phase of dispersal will increase knowledge of the dispersal process while providing important insights into population distribution of these insects in patchy landscapes.

Information is limited as to how red flour beetle detects habitat patches prior to physical contact. After a habitat patch is initially infested, beetles will aggregate in response to male - produced pheromones (Naylor 1961), but attraction to uninfested food has been reported to be weak (Barrer 1983, Phillips et al. 1993) and the influence of distance to the resource on detection is unknown. If red flour beetles respond to habitat patches using visual or olfactory cues prior to contact with the patch boundary, then this might influence how they locate new habitat in patchy landscapes. Because perceptual range is likely to be influenced by both exogenous and endogenous factors, I manipulated the following parameters to assess beetle response: distance of release point from patch, air movement, and hunger level, or lack of oviposition substrate. I hypothesize that, if beetles respond to patches before physically encountering the patch edge, the proportion of beetles locating the patch edge will be greater for patches with resource compared to those without resource, compared within each release distance. If a manipulated parameter (hunger level/lack of oviposition substrate, and air movement) is important in patch detection, then I should see a greater proportion successful in reaching the flour goal relative to its control (no starvation/oviposition substrate, no air movement), compared within each release distance.

To determine how differences in patch characteristics (quality) may influence beetle behavior and permeability of patches, I manipulated the amount of resource and shelter provided by a flour patch. I measured the permeability of patches by recording frequencies of transitions into and out of the patch as well as residence time in the patch. To further assess behavioral response to patch treatments, I calculated several metrics describing movement pathways in zones proximate to the patch and more distant. Because red flour beetles have been shown to preferentially visit and oviposit in larger amounts of flour rather than smaller (Campbell and Runnion 2003, Romero 2007a), I hypothesized that resource amount may be a factor in patch permeability. I hypothesized that if beetles prefer a larger amount of resource, then patches with a higher amount of resource will be more permeable than those with a lower amount of resource.

Patches can provide other resources than just food or oviposition sites. For instance, patches may also provide shelter and/or protection for both adults and progeny. Therefore, patch permeability may be influenced, not only by the amount of resource, but by other physical features that influence the quality of that patch. I predict that adding a shelter over a patch with a low amount of resource will increase permeability relative to both high and low resource treatment amounts by increasing the refuge quality of the patch. Because red flour beetles are often found moving along the edges of walls and machinery in flour mills and food-processing plants, edges of experimental arenas (Campbell and Hagstrum 2002), and flour patches (Romero 2007b), I also assessed the effect of patch and structural edges on beetle movement.

Methods

Perceptual range

To determine how distance, air movement, and hunger level/lack of oviposition substrate influence perception of habitat patches by female red flour beetle, I experimentally manipulated the above factors and used the number of individuals successfully detecting a flour patch (i.e., goal) in a wind tunnel as a measure of perception. The beetles' goal was designated as a line extending the width of the wind tunnel (82 cm) located 54 cm downwind from the air source (Figure 1). Upwind and adjacent to this goal line there was a 4 cm wide strip (the patch) extending the width of the wind tunnel which either contained wheat flour or had no flour as a control. For the treatment with flour, approximately 50 ml of flour was spread as evenly as possible over the strip area. Points at which beetles were released were located midway between the sides of the wind tunnel 2, 4, 8, or 16 cm from the goal. A line parallel to the goal was drawn 32 cm downwind from the goal line and designated as the exit line of the experimental arena. I chose 32 cm from the goal as the exit line because it was double the distance of the release point farthest from the goal (16 cm), thus making the distance from the release point to the exit line equal to the distance from the release point to the goal. Presence or absence of flour was tested as split-plot factors with air movement, hunger level/lack of oviposition substrate, and distance of release point from the goal as whole plot factors in a randomized complete block design.

Female beetles used in this study were taken from sub-colonies founded from individuals collected from a flour mill ~ 30 months before experiments were conducted. A single sub-colony was used for each experimental block. Each sub-colony from which beetles were used in the experiment had been initiated with ~ 50 adults and maintained in 0.25 L of wheat/brewers yeast mixture (95/5%) in an environmental chamber (27 ± 0.03° C; 56 ± 5% rh; 14:10 day/night cycle). Age was standardized by removing the initial adults after they had been allowed to mate and oviposit in the sub-colony for 4 days. This procedure standardized the age of the cohorts in each sub-colony so that all beetles were 3 weeks ± 4 days in age at the time of experiments. I removed beetles from a single sub-colony 24 h prior to use in the experiment, determined sex, and placed a group of 20-24 individuals in each of two 88 x 12 mm disposable polystyrene Petri dishes containing a piece of folded 70 mm diameter, grade 1 filter paper. Sex was determined by immobilizing beetles by chilling and observing with a microscope the absence of the setaceous patch present on the first femur of males (Good 1936).

For each distance category, beetles were assigned to a hunger level/lack of oviposition substrate and air movement treatment combination: they were either provided ~ 6.0 g of flour until the start of a trial (F) or kept without flour (starved) for 24 h beforehand (S). One half of each hunger level/lack of oviposition substrate group was tested in the wind tunnel with air flowing at a rate of 0.01 m/s (A) and the other half with no air flow (N). The four hunger/air treatments, FA, SA, FN, and SN combined with the four distance treatments resulted in 16 treatment combinations that were randomized within each split-plot. All whole plot combinations (16 with flour present + 16 with flour absent) were tested within a block on the same day between the hours of 12:00 and 19:00. The order of the split-plot was randomized within each block each day. Blocks were replicated over a 12 week time period totaling 32 replicates of each resource/distance/air/hunger combination. The floor of the wind tunnel was wiped clean with alcohol and lined with clean white paper between split-plots to eliminate odor cues from flour residue remaining from the flour treatment. Trials were conducted under ambient conditions (22.0 ± 10.0˚C; 21.0 ± 7.0% rh).

Two beetle release methods were used in experiments. In the first, an individual female beetle was held under a 1.5-cm diameter glass vial for a 90-s acclimation period at the appropriate release point before release into the experimental arena. I changed to a second release method after the initial 14 replicates in an attempt to reduce disturbance that appeared to trigger escape behavior by some beetles when the glass vial was removed. In the second method I used forceps to place the beetle in a paper “release envelope” in which it was sheltered but was able to leave at will. I constructed this envelope from two circular layers of filter paper, 3 cm in diameter, which were taped around the edges leaving four evenly spaced 5-mm exit holes. After comparing results of the first and second release methods, there were no significant differences in numbers of beetles reaching the goal, or in time to reach goal, so data were pooled for final analyses. After lifting the vial or when the beetle left the envelope, the beetle was observed until reaching the goal, reaching the exit line, or climbing and remaining on the side wall of the wind tunnel for more than 10 s. I stopped an observation after 10 min if not terminated earlier by one of the events mentioned above.

Behavioral observations of each beetle were recorded and the data organized using Observer software (Noldus Information Technology 2003b). I used Chi-square analysis of association (Pearson’s exact test, (SAS Institute, Inc. 2002) to test for difference in the number of beetles successfully reaching the goal with resource present or absent in pair-wise comparisons of all air/hunger combinations within a distance category. Chi-square analysis of association was also used to test for differences in numbers of beetles within a distance category (all treatments combined) either succeeding or failing to reach the goal (by exiting the arena, remaining on the side wall for > 10 s, or not reaching the goal within the 10 m observation period) with resource present or absent. Analysis of variance (ANOVA on ranks, GLM procedure, (SAS Institute Inc. 2002) was used to determine significant differences in the time taken to reach the goal in response to hunger level/ air movement treatments. For ANOVA, Tukey's hsd test was used for means separation at the alpha = 0.05 significance level.

Permeability of patch boundaries

To determine how patch quality may influence permeability, I manipulated patches using three treatments and examined various aspects of movement behavior in an experimental arena. Beetle response was measured in relation to the following patch treatments: a *high* amount of resource, a patch with 2.0 g of flour; a *low* amount of resource, a patch with 0.6 g of flour; and *sheltered*, a patch with a low amount of resource (0.6 g flour) with a flat piece of cardstock, the same dimensions (5 x 5 cm) as the patch, held 3 mm above the flour by an insect pin at each corner. The high-resource patch had more flour than the low-resource patch so that the edge of the high-resource patch was ~ 4 times higher than the edge of the low-resource patch (~1 mm and ~ 0.25 mm, respectively). The edge of the high-resource patch was laterally compressed so that, from the perspective of the beetle, it may have had the appearance of a wall of flour. The edge of the low-resource patch could be described as more of a gradient over which beetles appeared to walk easily. The covered patch had the same amount of flour as the low-resource patch, but a shelter was added. Although experimental arena was a simplified version of resources a flour beetle may encounter in a real landscape, the conditions simulate the types of matrix (bare concrete or wood floors) and patches that may be found in the anthropogenic environments in which red flour beetles thrive.

The experimental arena consisted of a 22.5 x 22.5 x 2.2 cm plastic polystyrene Petri dish bottom with a 1-cm layer of plaster of Paris covered by white bond paper that was secured on the edges by masking tape. Red flour beetles are not good climbers on smooth surfaces so were constrained to walk on the floor of the dish. I created a habitat patch in the center of the arena by sifting flour over a cardboard template with a 5 x 5 cm cut-out area and then removing the template. The insects were reared under the same conditions and standardized for age at 3 weeks ± 4 days as mentioned in the previous experiment. I removed beetles from a single sub-colony immediately before trials, determined sex, and placed females individually into the Petri dish arena to acclimate for 2 – 3 hours before the start of the trial. Beetles were free to explore the Petri dish arena; however, I kept beetles from contacting the patch during acclimation by placing a glass 88 x 12 mm Petri dish cover over each patch. Arenas with acclimating beetles were placed in an environmental chamber at 28° C at daylight setting until trials were run. The trials were conducted in a table-top environmental chamber at 28° C under 40 watt incandescent lighting. I placed arenas individually into the chamber, uncovered the flour patch, and recorded beetle movement for 10 min with a digital video camera. Four replicates of each of the three patch treatments were run in a series of trials in a complete randomized block design. There were 5 blocks run on sequential days, resulting in 20 replicates of each treatment.

To assess beetle response to patch treatments, I calculated several metrics of beetle movement pathways including: velocity; total distance traveled, a measure of general activity; and total sum of all turning angles, a measure of pathway complexity. Pathway metrics were calculated for three zones within the arena: 1) the patch edge zone (i.e., a 0.5-cm wide zone surrounding the patch 2) the matrix (i.e., a 7-cm wide zone between the patch edge zone and the structural edge zone), and 3) the structural edge zone (i.e. a 1.25-cm wide zone adjacent to the inside walls of the arena) (Figure 2). These metrics could not be calculated in the patch itself because movement could not be tracked due to the shelter over the patch or because beetles often tunneled into the flour in the high and low-resource treatments. To assess beetles' interaction with the patch, I calculated latency (time from occurrence of one event until the occurrence of a subsequent event) from the beginning of each trial until a beetle first entered the patch edge zone and first entered the patch zone. I measured permeability of patches as a result of patch treatments in several ways; I calculated latency from initial patch edge zone contact until first entering the patch as well as the frequency of transitions into and out of the patch. I determined the proportion of time spent in the patch as well as in the other zones. As an additional measure of permeability, I recorded whether or not the beetle entered the patch on the first encounter with the patch boundary. I also calculated cumulative frequencies of patch boundary interactions resulting in either entering or not entering the patch as well as those that resulted in the beetle exiting or not exiting the patch once inside. Data on the frequency of patch exiting were only collected for the high and low-resource treatments because it was not possible to observe the beetles in the sheltered patch. Ethovision behavioral tracking software (Technology 2003a) was used to calculate pathway metrics and other measures from the digital video recordings.

Analysis of variance (ANOVA on ranks, GLM procedure) was used to test means of behavioral measures for significant differences among main factors (patch treatments) and main factor x zone interactions. Tukey's hsd test was used to test for means separation at the alpha = 0.05 significance level. An arcsine-square root transformation (Zar 1999) was used to normalize proportions before statistical analyses. I used Chi-square test of association (Pearson's exact) to test for significant differences in distribution of frequencies of behaviors recorded at patch boundaries among patch treatments at the alpha = 0.05 significance level.

Results

Perceptual range

Within each release distance category, with flour present or absent, there were no significant differences in the number of beetles reaching the goal among pair-wise comparisons of all hunger level/air movement treatment combinations (2 cm – FA vs. FN, p = 1; SA vs. SN, p = 0.830; FA vs. SA, p = 0.532; FN vs. SN, p = 0.350; 4 cm - FA vs. FN, p = 0.381; SA vs. SN, p

= 0.174; FA vs. SA, p = 0.822; FN vs. SN, p = 0.496; 8 cm - FA vs. FN, p = 0.797; SA vs. SN, p = 0.778; FA vs. SA, p= 0.606; FN vs. SN, p = 1; 16 cm - FA vs. FN, p = 1; SA vs. SN, p = 0.1; FA vs. SA, p = 0.1; FN vs. SN, p = 1) (Figure 3). Because there were no significant differences among hunger level/air movement treatment combinations, data were pooled and the number of beetles succeeding or failing to reach the goal, with resource present and absent, was tested for significant differences within each distance category (n = 256, each distance category). As expected, I did see a decrease in the number of beetles reaching the goal with increasing distance of release points because, at distances closer to the patch edge, the probability of reaching the patch by chance is higher than at further distances, even when resource is absent. As the probability of reaching the patch, by chance, decreases then I expected to see more beetles reaching the patch with resource and fewer reaching the patch without resource. Results show no significant differences between numbers succeeding or failing with resource present or absent for beetles released at 2, 4, and 8 cm from the goal (Pearson's exact test, 2 cm, $\chi^2 = 0$, p = 1; 4 cm, $\chi^2 = 0.419$, p = 0.605; 8 cm, $\chi^2 = 0.449$, p = 0.528). However, at the 16 cm release point, there were significantly more beetles succeeding than failing to reach the goal with resource present than with resource absent ($\chi^2 = 9.004$, p = 0.003) (Figure 4).

As an additional measure of how the various parameters influenced beetles' perceptual range, I hypothesized that time for beetles to reach the patch will increase with distance, but that, within each distance category, starved/oviposition substrate deprived beetles with air movement will take less time to reach the patch edge than well-fed beetles with no air movement. Time to reach the goal increased significantly with increasing distance from the goal, as expected, (F = 22.41; df = 3, 368; p = <0.000), but there were no significant flour*distance interactions which would indicate beetles were responding to the presence of flour (F = 1.48; df = 3, 368; p = 0.2201). There was no significant effect of the split-plot factor (flour presence or absence) or the whole plot combination factor of hunger*air on time taken by beetles to reach the goal (ANOVA on ranks; flour, F = 0.07; df = 1, 368; p = 0.7853, hunger*air, F = 0.04; df = 1, 368; p = 0.8337). Blocks were significantly different among days of the experiment for time to reach the goal (F = 1.88, df = 23, 386, p = 0.009).

Response to patch boundaries

Beetles responded differently to patch treatments when moving in the overall arena, however, the greatest differences in movement parameters occurred at the patch edge and in the patch (Figures 5-8). Beetles moved much faster and straighter in the arena in the presence of a sheltered patch compared to both high and low-resource treatments. When shelter was present over the patches, the rate of movement in the overall arena was 28% faster (F = 5.79; df = 2,149; p = 0.0036) than when there were no shelters over the patches. When shelters were present over the patches the total distance moved by beetles in the overall arena was 1.6 and 1.5 times less than the high and low-resource patches without a shelter, respectively (F = 5.86; df = 2,149; p = 0.0036). The total sum of turning angles, a measure of pathway complexity, was 1.8 times greater in high and low-resource patches relative to the sheltered patch (F = 14.95; df = 2,149; p < 0.0001). There was a significant block effect for total distance moved (F = 2.65; df = 4,149; p = 0.0354), but no significant block effect for velocity (F = 1.75; df = 4, 149; p = 0.1419) nor total angles (F = 1.92; df = 4,149; p = 0.1106).

There were no significant interaction effects of either structural edge or matrix zones with any patch treatment, indicating that the influence of the patch did not extend to those areas of the experimental arena. The significant interaction effect of patch edge zone with patch treatment was the main source of variation in movement parameters (zone x patch resource amount treatment). Thus, the greatest influence on beetle movement due to patch treatments occurred as beetles approached the patch edge (velocity, F = 6.05; df = 4,149; p = 0.0002; total distance, F = 2.83; df = 4,149; p = 0.0268; total angles, F = 5.24; df = 4,149; p = 0.0006; Figure 5A - C).

Beetle movement at the patch edge was impacted by the patch treatment in different ways. Beetles moved 3 times faster at the patch edge of the sheltered patch than at the patch edge of both the high or the low-resource patches (shelter vs. high, p = < 0.0001; shelter vs. low, p = 0.0002) (Figure 5A). Beetles moved at similar rates of speed at patch edges of high and low-resource treatments (p = 1.00). The total distance moved by beetles at the patch edge of the high resource patch was similar to the distance moved in the patch edge zone of the low- resource patch (p = 0.092), but significantly greater than at the edge of the sheltered patch (p = 0.011). Distances moved in the patch edge zone of the sheltered and low-resource patches were not significantly different (p = 0.999) (Figure 5B). The total sum of turning angles was 10 times greater at the patch edge of the high resource patch compared to the sheltered and 4 times greater compared to the low-resource patch (high vs. sheltered, p = < 0.0001; high vs. low, p = 0.037), indicating a more complex pathway at the edges of high-resource patches and a more linear pathway at the edges of the sheltered and thin-resource patches. There were no significant difference in total sum of turning angles in the patch edge zone of the sheltered and low-resource patches (p = 0.134) (Figure 5C).

Latency to contact the patch edge zone was similar among patch treatments (treatment effect, F = 1.46; df = 2, 42; p = 0.2437). Latency to contact the patch was also similar among patch treatments (F = 1.69; df = 2,42; p = 0.1982). However, the time it took a beetle to enter the patch after first contacting the patch edge zone was significantly less in the presence of a sheltered patch compared to the high and low-resource treatments (block effect, F = 1.16; df = 2,39; p = 0.3442, treatment effect, F = 6.46, df = 2,39, p = 0.0038) (Figure 6).

There were no zone x patch treatment interactions for number of transitions into a particular zone and thus, no significant differences in the number of transitions into the patch among treatments (F = 1.54, df = 6,219, p = 0.166) (Figure 7A) however, beetles spent proportionally different amounts of time in the patch depending on patch treatment. Time spent in covered and high-resource patches was similar (p = 0.999) and beetles stayed longer in these than in low-resource patches, a significant difference of 39% and 26%, respectively (sheltered vs. low, p = 0.0015; high vs. low, 0.024) (Figure 7B). Beetles spent statistically similar proportions of time in the structural edge and patch zones, 45% and 36%, respectively (p = 0.557) (Figure 8). Although the matrix occupied 71% of the area in the experimental arena, beetles spent significantly less time in that area than at structural edges which occupied only 22% of the total area (structural edges vs. matrix, p = 0.006). Time in the matrix was statistically similar to the time spent in the patch (patch vs. matrix, p = 0.256). Beetles spent 6% of the time at the patch edge, significantly less time than in the other zones (structural edge, matrix, and patch vs. patch edge, p = < 0.0001). Time spent at the structural edge, in the matrix, and in the patch was not proportional to the area occupied by the various zones (Figure 8). Only the proportion of time spent in the patch edge zone was relatively consistent with expectations based on the area of the zone. Beetles spent considerably more time at the edge of the high-resource patch, 13%, than at the edges of the sheltered patch (high vs. sheltered, p = 0.01). Beetles spent 1% of the time at the edge of the sheltered patch, statistically similar to the 3% spent at the edge of the low-resource patch (sheltered vs. low, p = 0.962). Even though beetles spent more time at the edge of the high-resource patch, the amount was not significantly different than at the low-resource patch (p = 0.446) (Figure 9). Time spent at the structural edges and in the matrix were not significantly different among patch treatments (structural edge - shelter vs. high, p = 0.997, shelter vs. low, p = 0.776, high vs. low, p = 0.998; matrix – shelter vs. high, p = 1.0, shelter vs.

low, $p = 0.570$, high vs. low, $p = 0.2481$, data not shown) indicating that patch treatments did not impact the proportion of time spent in areas of the experimental landscapes other than the patch or the patch edge.

Observation of beetle behavior at the patch boundary further revealed that beetles perceived the patches differently and that their perception impacted permeability. There were significant differences in the proportions of beetles entering or not entering the patch on the first encounter among patch treatments (Pearson's exact test, $\chi^2 = 17.799$, df = 2, $p < 0.0001$) (Figure 10). On the first encounter, no beetle entered the patch in the high-resource treatment compared to 50 and 60% for the low-resource and sheltered treatments. These differences are indicative of the distinct contrast in beetles' initial response to these patches, showing that, on the first encounter, patches with a low amount of resource or a shelter were significantly more permeable to beetle movement from the matrix to the patch than patches with a high resource amount. However, when cumulative encounters with boundaries were calculated, permeability changed when beetle were both entering (Pearson's exact test, $\chi^2 = 16.677$, df = 2, $p = <0.0001$) and exiting a patch (Pearson exact test, $\chi^2 = 16.677$, df = 2, $p = <0.0001$) (Figure 11). For boundaries approached from the matrix, only 47% of the total cumulative encounters with a high-resource patch resulted in a beetle entering a patch compared to the much higher probabilities (61 and 84% respectively) of entering low-resource and sheltered patches. For boundaries approached from the patch interior, there was approximately a 50/50 probability that an encounter with the high-resource patch boundary would result in a beetle leaving a patch, once inside, compared with a higher probability (79%) that an encounter would result in a beetle leaving a low-resource patch. With subsequent encounters permeability of all boundaries from the matrix to the patch increased, however the high-resource patch did not reach the high permeability levels of the sheltered or the low-resource patches.

Discussion

Knowledge of perceptual range in red flour beetle is vital for understanding movement patterns and population distribution in these insects. I hypothesized that, if beetles respond to a habitat patch before physical contact, there should be a greater proportion of beetles orienting toward and reaching a goal containing flour resource than reaching a control patch containing no flour resource. I also predicted that the starvation/lack of oviposition substrate x air movement treatment may increase the ability of beetles to detect a flour resource. Results of this study generally do not support these hypotheses, instead indicating that red flour beetle does not exhibit directed orientation to habitat patches prior to contact and that hunger/lack of oviposition substrate and air movement do not enhance their response to habitat. I observed a decrease in the proportion reaching the goal with increasing distance, as predicted, due to the decrease in the probability of reaching the goal by chance as the goal gets farther away from the release point. This and a previous study examining movement behavior in experimental landscapes (Romero 2007b) lend support to the premise that red flour beetle do not detect patches until a systematic search strategy brings them in physical proximity with the patch edge. If their perceptual resolution is as restricted as it appears to be, red flour beetles may have limited information about the pattern of resources as they move about in a landscape. Thus, they may only assess the quality of patches through direct physical contact. This suggests that the best dispersal strategy for a red flour beetle may be to colonize the first acceptable patch encountered because further dispersal for an animal with a narrow perceptual range could be quite risky. If the probability of encountering suitable patches in a landscape is high without prior detection, there may be little selection for the ability to detect patches before physical encounter. Red flour beetles have been characterized as a colonizing rather than a competitive species (Dawson 1976) and thus, even though perceptual range may be limited, they appear to have evolved movement rules that result in colonizing episodes leading to successful dispersal.

It is possible that beetles showed no directed orientation to the flour because odor cues from the flour so permeated the experimental area that there was no directional information beetles could use for orientation. Beetles appeared to exhibit directed orientation to the resource from the 16 cm release point, suggesting that, at this distance, volatile cues may have been used to find the flour resource. However, because there is no consistent pattern in the remainder of the data, this result must be interpreted cautiously. Additionally, beetles as close as 2 or 4 cm from the flour could have perceived the patch visually or chemically, but were not motivated to make contact with the patch. It is certainly possible that beetles could perceive the flour resource, but were constrained by some other behavior not associated with finding the resource such as searching for shelter. These results support other research indicting that red flour beetles show limited orientation to food volatiles (Barrer 1983, Phillips et al. 1993). I do not rule out the influence of other exogenous or endogenous factors not tested in my study which may serve to increase motivation of beetles to find habitat patches. A suggestion for future studies of habitat perception in red flour beetles is to focus on how physiological factors such as extended starvation (energy depletion), and egg load may increase motivation to detect habitat. Additionally, based on other work (Romero 2007a) I suggest that beetles motivation to find patches may change the longer they are engaged in exploratory behavior so future work should include manipulation of the temporal window in which beetles may locate habitat.

If red flour beetles have a limited perceptual range, then how do they reduce risks and increase dispersal success in patchy landscapes? The use of one or a combination of systematic search strategies may mediate dispersal risks associated with small perceptual range. But, even when certain strategies can be identified, such as ranging or area restricted search, these behaviors may interact with landscape structure producing movement patterns idiosyncratic to the species in question. Therefore empirical data, elucidating how a focal organism perceives and interacts with structures of the landscapes it inhabits, are clearly needed to better indicate how a particular landscape may function.

To determine if beetles are able to evaluate patches before physical contact, I focused on how permeability of patch boundaries might differ in response to treatments manipulating patch quality. My results show that red flour beetles evaluated patches differently depending on resource amount and shelter, but that evaluation may not occur until there is physical contact with the patch. Manipulation of resource amount and shelter resulted in considerable differences in patch boundary permeabilities at the first encounter. Treatments also resulted in differences in patch permeability that changed when cumulative encounters were considered. Movement pathways of insects have been shown to be sensitive to landscapes structure (Jonsen and Taylor 2000, Goodwin and Fahrig 2002), including boundaries (Ries and Debinski 2001, Collinge and Palmer 2002, Haynes and Cronin 2006), indicating differences in perception of landscape structure. In this experiment, metrics of red flour beetle movement pathways showed significant differences only at the interface, or ecotone, between the matrix and the patch, indicating that perception of the ecotone was quite different among treatments. Higher velocity, shorter latency, shorter travel distance, and lower total sum of turning angles in the ecotone of the sheltered patch all indicate that beetles entered the sheltered patch very quickly and directly compared to the high and low-resource treatments. Alternatively, lower velocity, longer latency, higher travel distance, and higher total sum of turning angles indicate that beetles lingered longer and their pathways were more complex in the ecotone of the high-resource patch compared to the other two patch treatments.

I proposed several hypotheses concerning the impact of patch treatments on permeability. First, I hypothesized that, if beetles prefer a higher amount of resource, then high-resource patches will be more permeable than low-resource patches. Secondly, I predicted that low-resource patches may be more permeable due to a lower edge (smoother gradient) between the area surrounding the patch (matrix) and the patch itself. Boundary transition data generally do not support my first hypothesis but do support the second concerning relative permeabilities of the high and low-resource patches. Permeability of the high-resource patch was relatively low compared to the low-resource patch at the first encounter with the patch, but, with subsequent encounters, permeabilities of the two became more equal.

Once beetles were inside the patch, the difference in probabilities of not exiting the patch became larger between the high and low-resource patches, 50 vs. 20% respectively. Beetles more frequently exited the low-resource patch which resulted in higher permeability. The differences in probabilities of exiting the patches resulted in a directional bias in permeability (Wiens 1992) of the patch-to-matrix boundary for high relative to low-resource patches. The net result is that 25% of boundary encounters result in beetles remaining inside high-resource patches compared to 12% of boundary encounters resulting in beetles remaining inside low-resource patches. The decrease in exiting high-resource patches resulted in increased patch residence time in high compared to low-resource patches. Observations that permeability changes after beetles enter the high-resource patches and that residence times were higher in the high-resource patch supports my hypothesis that evaluation of patch quality is made after contact with the patch boundary, and especially after entering the patch.

There may be several interacting explanations for the increased difference in the permeability of the patch-to-matrix boundary between the high and low-resource patches. Once inside the high-resource patch, the higher viscosity (resistance to movement) of the deeper flour may have retained beetles; the ‘hardness” of the compacted edge could have impeded movement over the boundary; or beetles may have preferred the high-resource patch because it provided more resource and shelter than the low-resource patch. Data presented here suggest that the “hardness” of the compacted edge of the high-resource patches may have elicited very different responses among individual beetles because, while not significantly different from the low-resource patch, latency from contact with the ecotone until entering the patch for the high-resource patch showed substantial variation. Moreover, a measure of the complexity of beetle movement pathways, the metric ‘total angles’, indicated that movement pathways in the ecotone of the high-resource patch were significantly more complex than in the ecotones of the other patch treatments. On first encounter, it appears that the high-resource patch was perceived by beetles as less permeable, than the low-resource boundary. This was most likely due to the height and compaction of the edge of high-resource patch, which seemed to encourage beetles to move along the edge instead of enter the patch. These observations could have important consequences for patch colonization by an insect with a limited perceptual range in real landscapes that are not closed systems (as was my experimental arena). In an open system, if red flour beetles do not enter a high-resource patch on the first encounter in response to some other patch characteristic besides resource amount, and continue searching the landscape, they may possibly lose contact with the patch. In this case, colonization of a potentially high quality patch may not occur. Conversely, if beetles make return visits to high quality patches, eventually crossing their boundaries, then directional permeability and preference may lead to aggregation in these patches.

My third hypothesis concerning patch quality was that permeability would increase in sheltered patches relative to both high and low-resource treatments because adding a cover would increase the refuge quality of the patch. My results clearly support the prediction that the enhanced refuge quality of the patch increases permeability. As stated above, beetles entered the sheltered patch very quickly and directly compared to the other patch treatments. Residency time was comparable to the high-resource patch so these patches were clearly preferred by beetles over low-resource patches. At first encounter, the probability of entering a sheltered patch was 60%, slightly higher than the low-resource patch, which was quite permeable at 50%. The large difference in cumulative probabilities of entering the sheltered patch or not, 84 vs. 16%, indicate both the high permeability and strength of attraction of the sheltered patch for red flour beetles. Total boundary encounters for sheltered patches were 38% lower than for high-resource patches and 15% lower than the low-resource patches, indicating that beetles left and re-entered the other patches more frequently. These observations coupled with long residency time strongly indicate that the sheltered patches were the most preferred of all patch types even though the amount of resource contained in the patch was less than the high-resource patch and similar to the low-resource patch. Why beetles would prefer a patch with less rather than more resource may be explained by the sheltered patch having a lower light level, or some other characteristic due to the presence of the cover, that was more attractive to the beetles.

Evidence provided by my other studies indicates that preference for shelter could, however, change over time. A study of red flour beetle movement and oviposition response in experimental flour patch landscapes (Romero 2007a) indicated that beetles appeared to be engaged in an initial exploratory phase after release into new landscapes and that oviposition was likely delayed for ~ 24 h. If beetles were in an exploratory phase in the current study, they may not have been following cues related to foraging or selecting reproductive sites and rather were more interested in finding shelter. Later, after the initial exploratory phase, beetles' responsiveness to cues associated with reproductive site selection may come into play and preference for resource amount may then take precedence over shelter. In any case, the amount of resource in patches in the current study was more than sufficient for beetle reproduction (Campbell and Runnion 2003) and so patches with low resource amount and shelter still may be preferred over those with no shelter and higher resource amount as beetles begin laying eggs. I suggest that preference for shelter should be examined at different time points during dispersal as perception of patches may change with both activity and exploration time.

Results of this study indicating that the quality of a patch as shelter may take precedence over other habitat patch characteristics is similar to that seen during a radio-tracing study of movement of a carabid beetle, *Alba parallelepipedus*. This beetle covered more area moving in hedgerows, which were more resistant to their movements, rather than in open areas less resistant to movement, presumably because hedgerows provided increased protection (Charrier et al. 1997). In another dispersal study, Natterjack toadlets (*Bufo calamita*) preferred to move in forested environments that were highly resistant to their movements rather than in more open areas with less resistance, presumably because forests provided increased protection from predators and desiccation (Stevens et al. 2004). Therefore, as individuals are moving among patches on real landscapes, preference for shelter may take precedence over resource amount or other patch characteristics deemed to be correlated with patch quality because this behavior may reduce risk.

The propensity of beetles to linger at and to have more encounters with edges of high-resource patches lends support for my hypothesis that beetles tend to aggregate at patch edges. It appears that high-resource patches, because of their high, compacted edge, elicited a thigmotactic response in the beetles similar to the edge of a structure. These results also support other observations reported in this dissertation (Romero, 2007b) showing that beetles moved more slowly and in a more complex manner in experimental landscapes with a high number of habitat patch edges. Beetles spent twice as much time on the edges of the experimental arena (structural edge zone) than in the matrix even though the size of the matrix zone was three times larger than the size of the structural edge zone. My results, concerning edge-following, coincide with those of Campbell and Hagstrum (2002) who observed that red flour beetles had a tendency to be inactive, to move along, and to move more slowly at edges of an experimental arena. I propose that both structural and habitat edges may increase aggregation of beetles in areas of high edge concentration.

The task of linking fine-scale movements to population-scale distribution patterns is a daunting task indeed (Wiens et al. 1993, Turchin 1998) due to high variability in animal movement responses and the problem of translating results across scales. However, empirical studies such as mine provide much needed information on how movement behavior interacts with landscape structure. The information presented here is an important step to understanding how a focal species may be distributed on real landscapes. By combining detailed movement pathway analyses with measures of boundary permeability, I was able to gain new insights concerning how red flour beetle interact with patch boundaries that may be used to predict population distribution patterns. My study highlights how permeability may change with subsequent encounters as individuals gain more information on patch quality as well as how shelter may increase permeability. Behaviors, such as these, could be very important in controlling the flux of beetle movement among patches. I contend that movement models should consider that response to habitat boundaries may change with time and experience and consider, also, that preference for shelter and propensity to aggregate along habitat and structural edges may profoundly impact the distribution of individuals.

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Figures

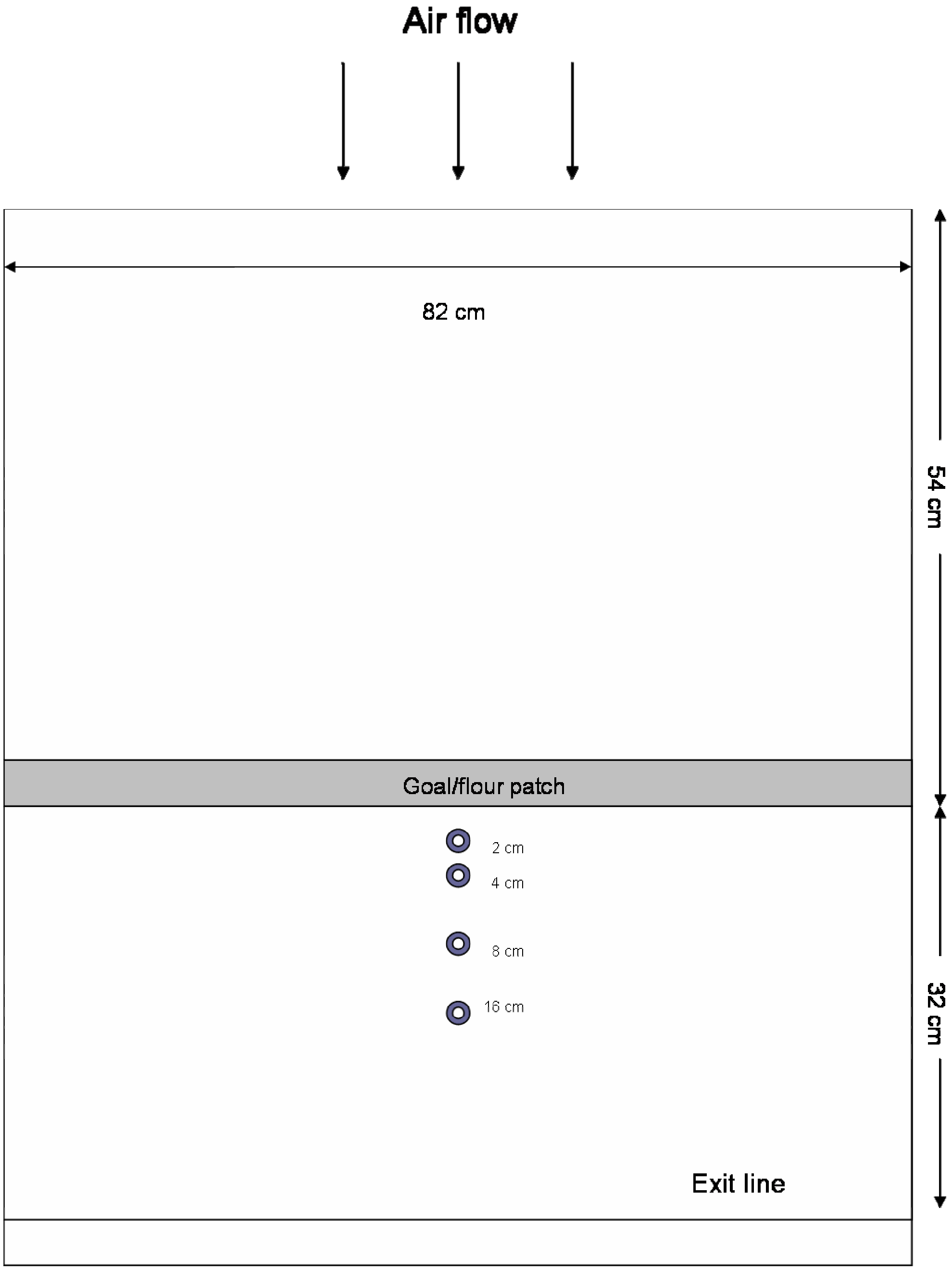

Figure 3-1 Diagram of the experimental arena in the wind tunnel. Female red flour beetles (*Tribolium. castaneum*) were released at labeled points 2, 4, 8, and 16 cm downwind from the goal/flour patch.

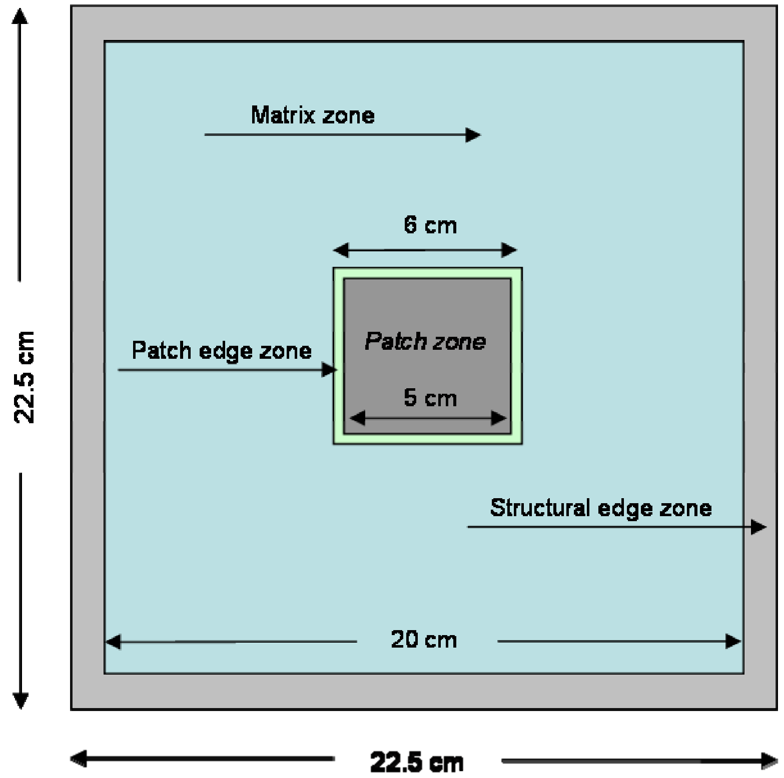

Figure 3-2 Experimental arena with various zones used to observe red flour beetle (*T. castaneum*) response to three patch treatments designated as: *High* amount of resource, a patch with 2.0 g of flour and a 2 mm high edge, *Low* amount of resource, a patch with 0.6 g of flour and a low edge ~ 0.5 mm, and *Shelter*, a low resource (0.6 g) patch covered by a 5 x 5 cm flat cardboard shelter supported 3 mm above the flour surface.

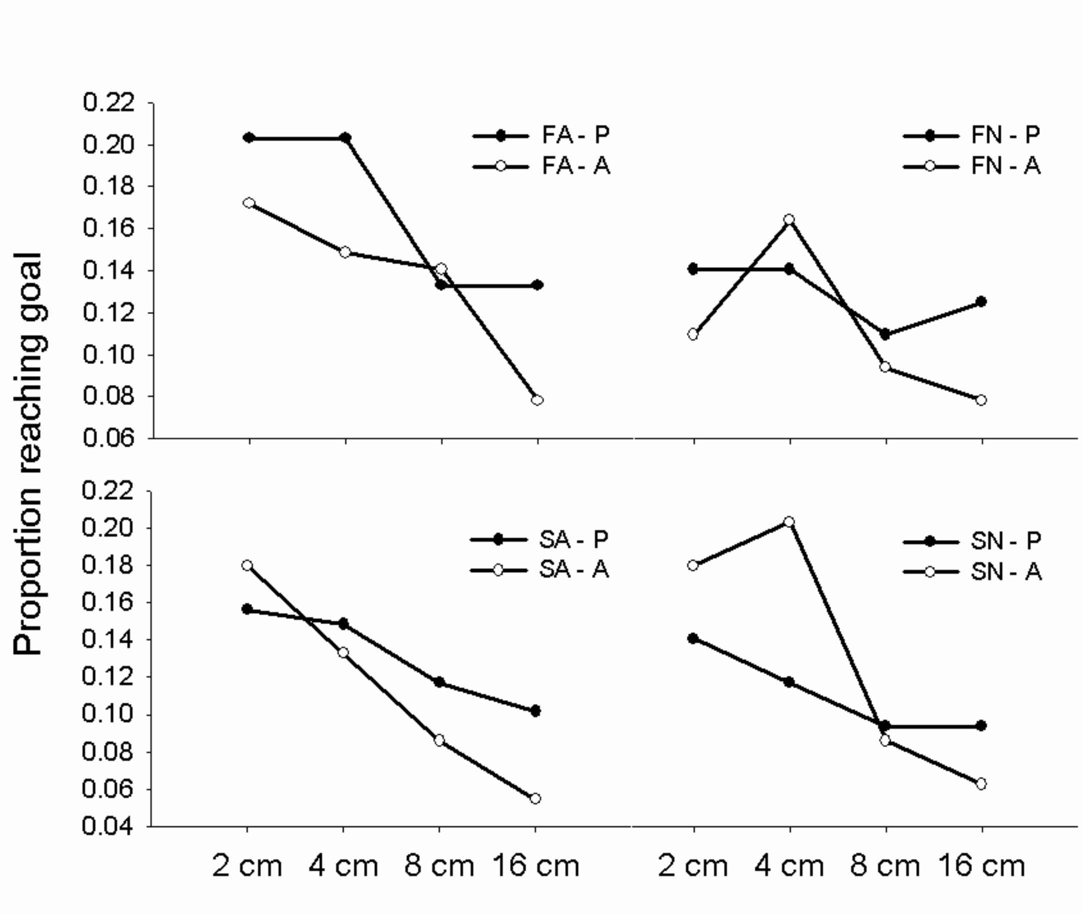

Figure 3-3 Proportion of female red flour beetles (*T. castaneum*) reaching a goal with flour resource present (P) or absent (A) by walking from distances of 2, 4, 8 and 16 cm. Responses are in relation to hunger status and air movement treatment combinations including: fed (exposed to food until start of experiment) with air movement (FA), fed with no air movement (FN), starved 24 h with air movement (SA), and starved 24 h with no air movement (SN). Proportions were calculated by dividing the number of successes by the total number of observations (n = 128) per each distance, hunger status, and air movement treatment combinations for trials with either resource present or absent. Chi –square analysis of association (Pearson exact test) comparing number of beetles reaching the goal, with resource present or absent, shows no significant differences among all pair-wise comparisons of above treatments within a distance category.

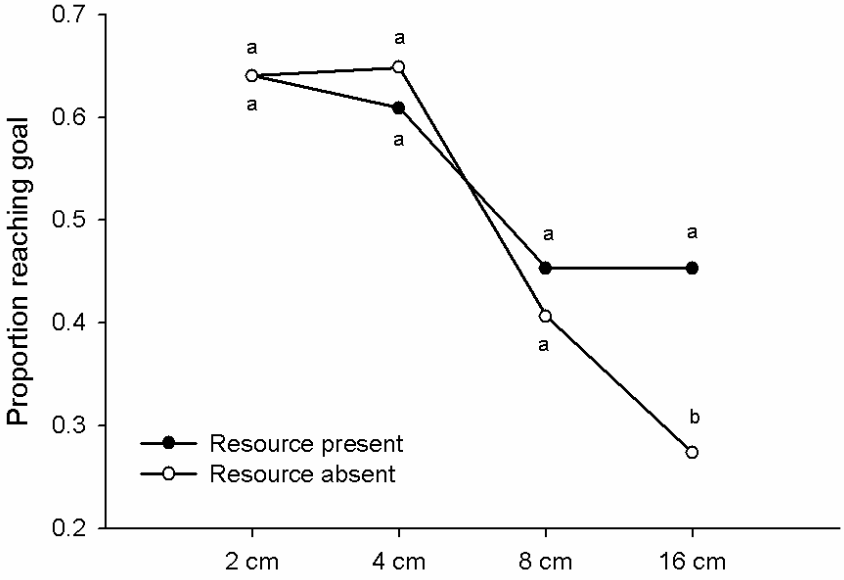

Figure 3-4 Proportion of female red flour beetles (*T. castaneum*) reaching a goal with flour resource present or absent by walking from distances of 2, 4, 8 and 16 cm. Proportions were calculated by dividing the number reaching the goal by the total number of observations (n = 128, hunger status and air movement treatments combined) with resource either present or absent. Chi – square analysis of association (Pearson exact test, alpha = 0.05) shows no significant differences between number of beetles succeeding or failing to reaching the goal at distances of 2, 4, and 8 cm with resource present or absent, but a significant difference at the 16 cm distance (hunger status and air movement treatments combined, failures to reach goal not shown).

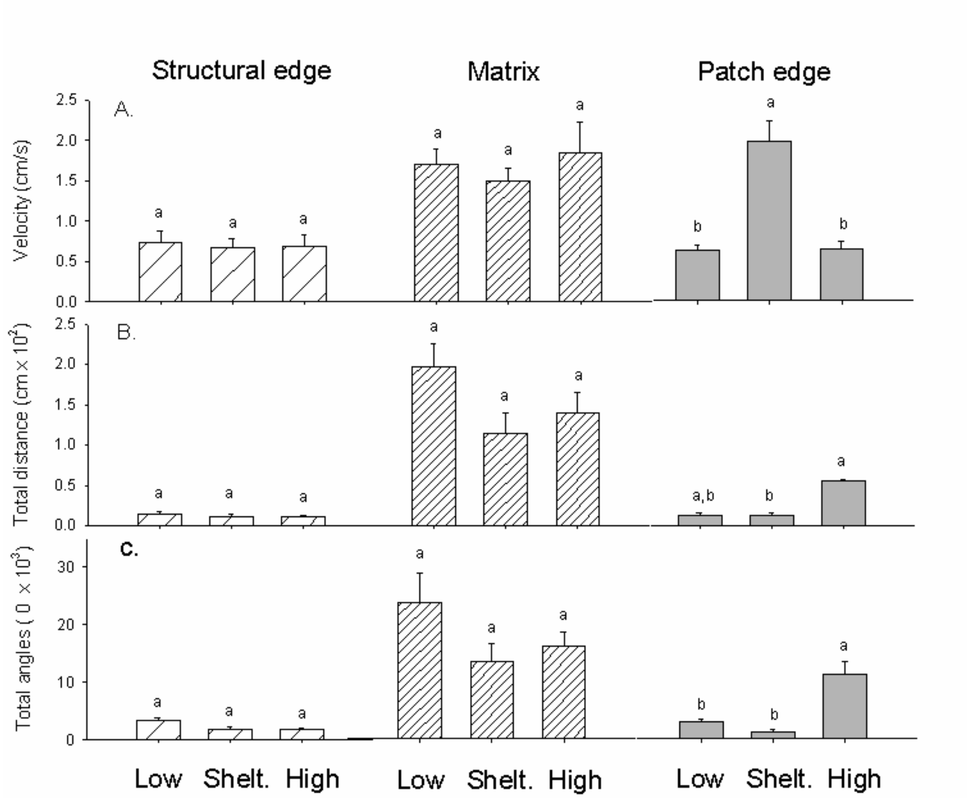

Figure 3-5 Mean + SEM of A.) velocity, B.) total distance moved, and C.) total turning angles of red flour beetle (*T. castaneum*) pathways in various zones of the experimental arena in response to flour patch treatments low resource, shelter, and high resource. Bars within a plot with the same letter are not significantly different (ANOVA on ranks, GLM procedure, alpha = 0.05).

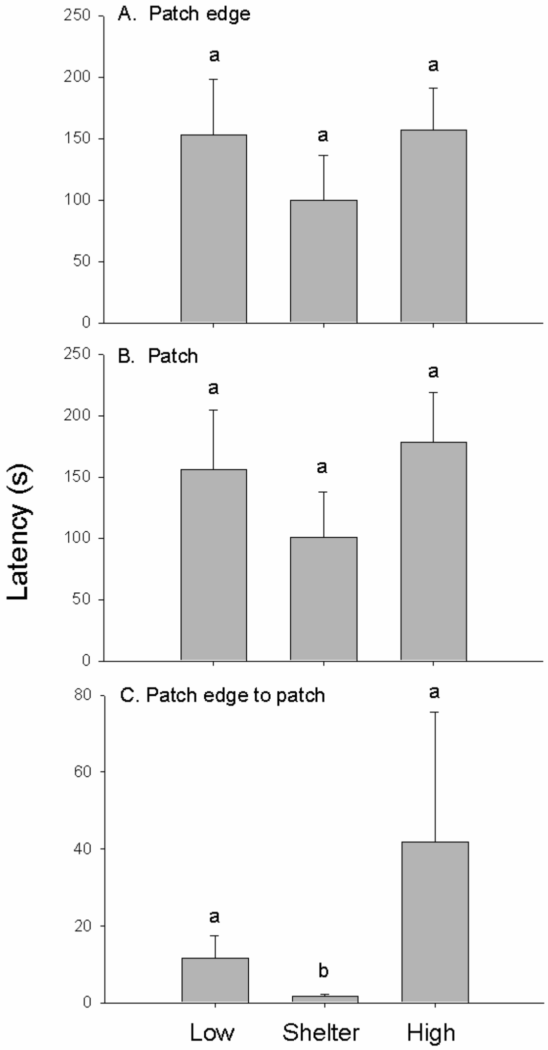

Figure 3-6 Mean + SEM for latency (time) from start of experiment for red flour beetle (*T. castaneum*) to enter (A.) patch edge and (B.) patch zones, and to enter (C.) patch after reaching patch edge zone in response to patch treatments, low resource, shelter, and high resource. Bars within a plot with the same letter are not significantly different (ANOVA on ranks, GLM procedure, alpha = 0.05).

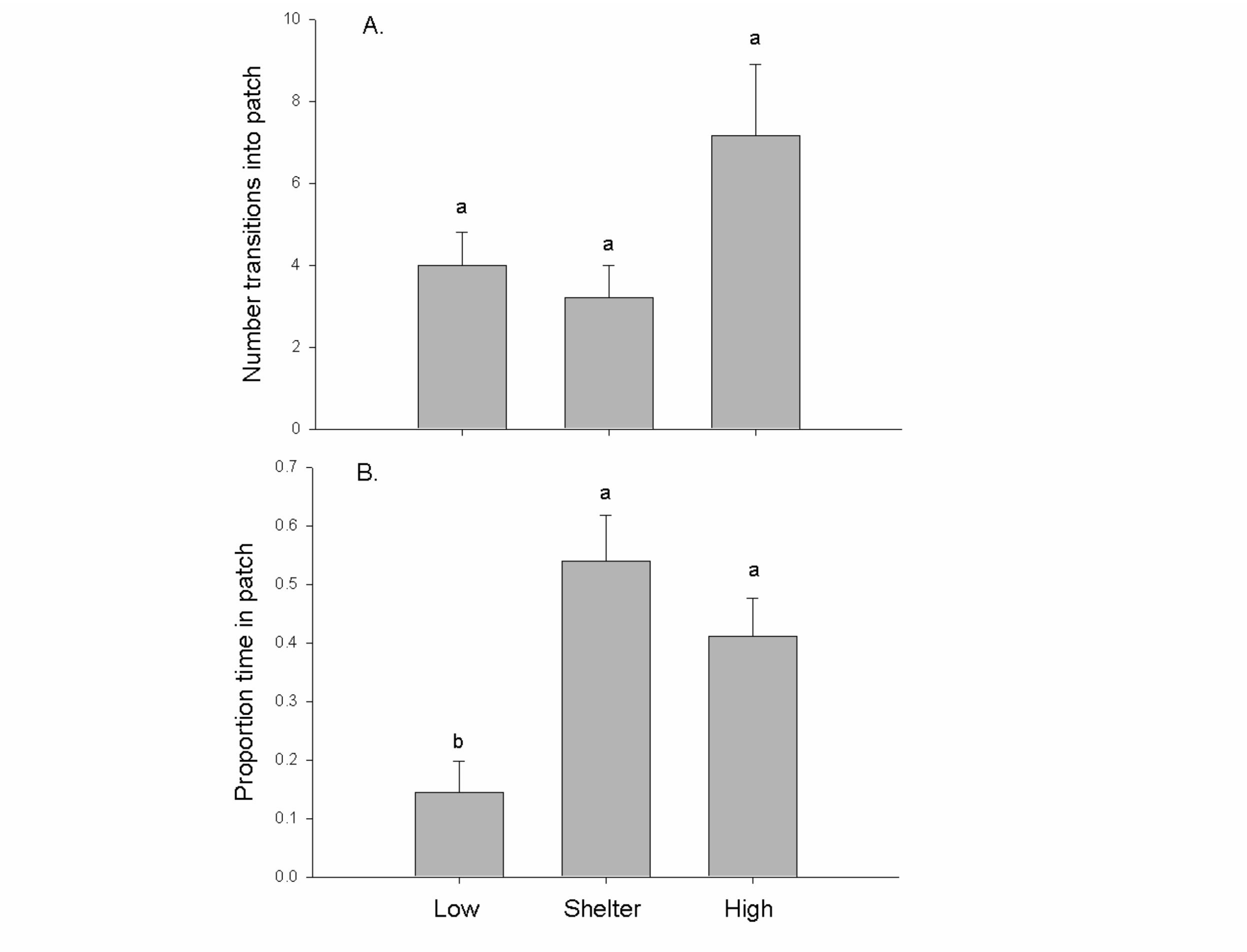

Figure 3-7 Mean + SEM of (A.) number of transitions into the patch zone and (B.) proportion of time spent in patch zone by red flour beetle (*T. castaneum*) in response to the three patch boundary treatments of low resource, shelter, and high resource. Bars within a plot with the same letter are not significantly different (ANOVA on ranks, GLM procedure, alpha = 0.05).

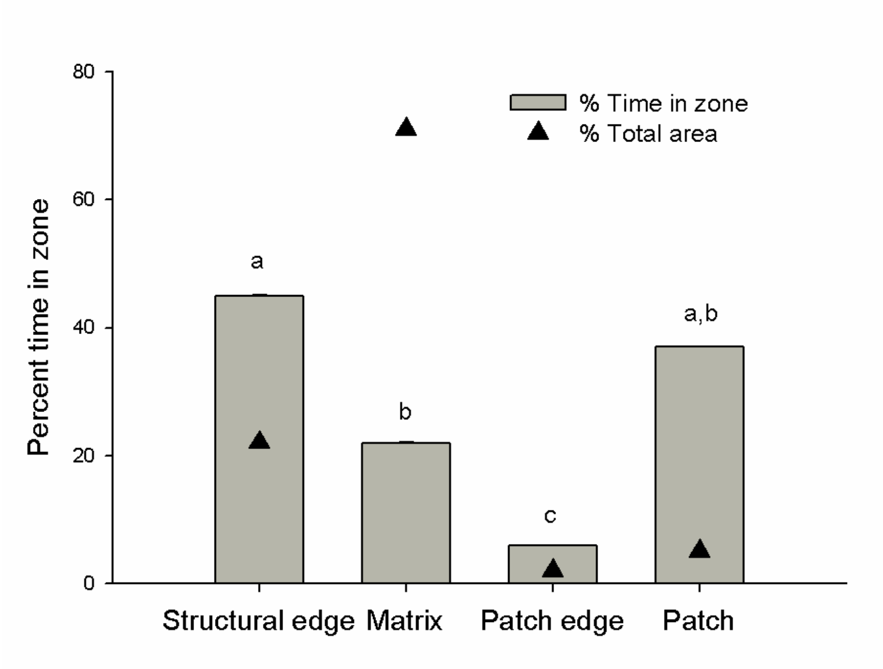

Figure 3-8 Mean ± SEM for percent of time red flour beetle (*T. castaneum*) spent in various zones of the experimental arena in relation to percent of the area occupied by the respective zone. Bars with same letters are not significantly different (ANOVA on ranks, GLM procedure, alpha = 0.05).

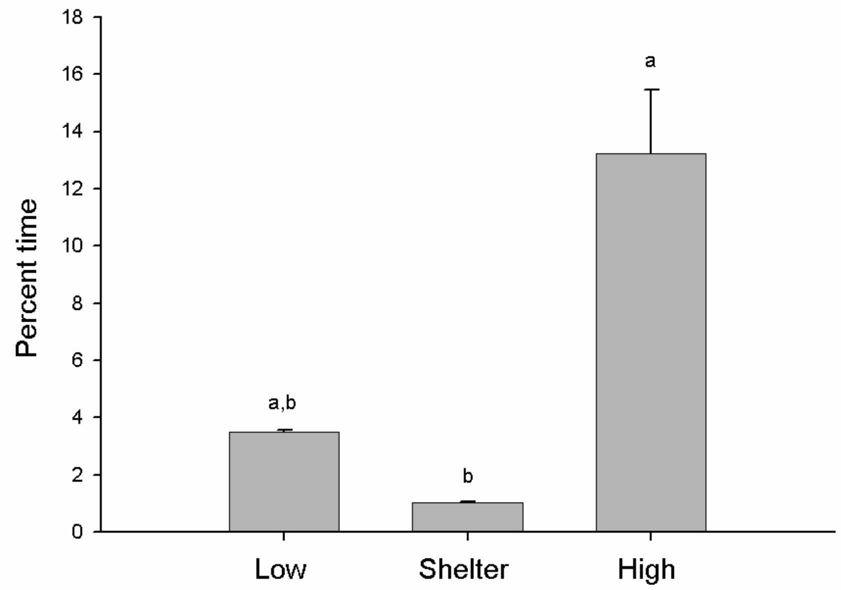

Figure 3-9 Percent ± SEM of total observation time (10 min) spent by red flour beetle (*T. castaneum*) in the patch edge zone in relation to patch treatments of *low* resource amount, *shelter* with low resource amount, and *high* resource amount. Bars with same letters are not significantly different (ANOVA on ranks, GLM procedure, alpha = 0.05).

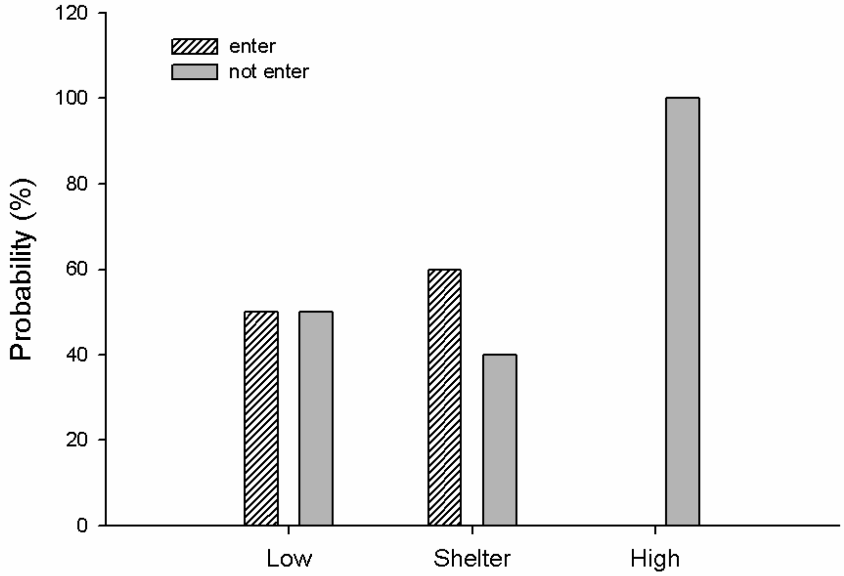

Figure 3-10 Probability that red flour beetle (*T. castaneum*) will or will not enter a patch on the first encounter in relation to the patch treatments of *low* resource amount, *shelter* with *low* resource amount, and *high* resource amount. Chi-square analysis of association shows distribution of probabilities among patch treatments to be dissimilar (Pearson's exact test, alpha = 0.05, n = 20 per treatment).

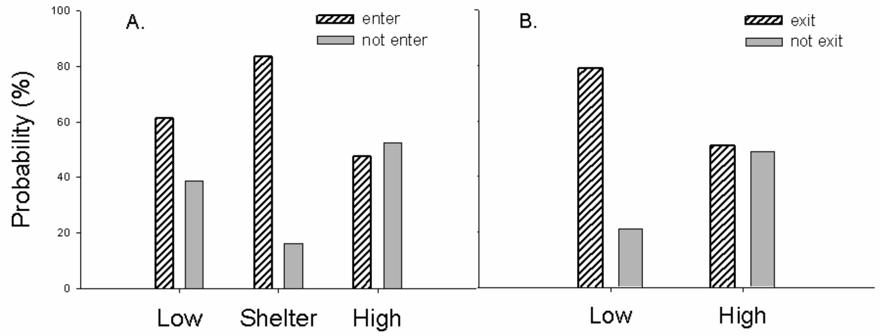

Figure 3-11 Probabilities that red flour beetle (*Tribolium castaneum*) will A.) enter or not enter and B.) exit or not exit the patch based on the patch treatments of *low* resource amount, *shelter* with low resource amount, and *high* resource amount. Probabilities are based on total number of encounters during a 10 min observation period for each patch treatment category (Plot A. Low – n = 57, Shelter – n = 49, High – n = 78; Plot B. Low – n = 38, High – n = 53).

CHAPTER 4- Functional Response of Red Flour Beetle (*Tribolium castaneum*) to Landscape Structure: Movement and Oviposition

Abstract

There is limited information concerning the spatial scales at which insects perceive habitat patchiness and how this perception influences the functional use of landscapes. This study used lacunarity analysis (index describing the variability of gap sizes among locations) to compare space use by red flour beetle (*Tribolium castaneum*) during movement and oviposition in landscapes with equal amounts of resource, but three levels of habitat fragmentation: clumped, intermediately fragmented, and highly fragmented. Lacunarity analysis showed that the pattern of movement was similar to the habitat pattern, but oviposition sites were significantly more aggregated than both beetle movement and underlying habitat pattern. Oviposition appeared to have been delayed for ~ 24 hrs following an initial exploratory phase that was characterized by increased movement. Although the total number of eggs was not different among landscapes, there were differences in the spatial arrangement of cells (locations) with eggs and the number of eggs per cell depending on the level of habitat fragmentation. Beetles laid significantly more eggs in fewer cells in fragmented landscapes compared to clumped landscapes. In intermediately-fragmented and clumped landscapes, beetles laid significantly more eggs in previously visited cells, but on fragmented landscapes females did not exhibit this behavior. Beetles preferentially oviposited in larger patches in fragmented and intermediate landscapes. These results suggest that choice of oviposition sites is influenced by a complex set of factors including previous exploration, resource amount, and scale of patchiness.

Introduction

Dispersal plays a fundamental role in ecology and evolution of animals, influencing intra- and interspecific interactions and population spread, dynamics, and genetic structure (Hanski and Gilpin 1997, Tilman and Karieva 1997, Turchin 1998). Information on movement is useful for development of population models and pest management practices (Stinner et al. 1983, Turchin 1998, Russell et al. 2003). Many theoretical advances have been made on the subject of optimal host selection by dispersers, especially in areas of plant-insect (Jaenike 1978, Thompson 1988) and host-parasitoid interactions (Godfray 1994). Resource selection for oviposition in herbivorous insects has been modeled with assumptions of equal availability and encounter rates of food plants (Singer 1986, Thompson and Pellmyr 1991) and ability for adult females to rank resources according to the relative fitness they provide to offspring (Mangel 1987, Ward 1987). However, these assumptions have rarely been supported empirically (Ward 1987, Mayhew 1997). More recently, how dispersers perceive and respond to landscape structure has been the subject of theoretical and empirical research (With et al. 2002, Zollner and Lima 2005). Species-centered empirical studies of movement behavior in landscapes are especially valuable for determining habitat use because behavioral response to the scale of patchiness determines, in part, the probability of habitat selection for animals foraging for food or sites for reproduction (Kareiva 1982).

Landscape structure can influence habitat selection (O'Neill et al. 1988, Wiens 1989) and thus influence evolution of dispersal (Baguette and Van Dyck 2007) and oviposition strategies (Thompson 1988), and parent-offspring conflict (Roitberg and Mangel 1993). Over the last two decades, experimental model systems have be used to examine the movement response of insects to spatial heterogeneity and to assess species' perception of landscape structure (Wiens et al. 1993). These models have become valuable tools for the investigation of the effects of landscape structure on ecological processes (Andren 1994, Schumaker 1996, Wiens et al. 1997, With et al. 1997). Search strategies of insects in response to landscape heterogeneity have received much attention over the last few decades (Bond 1980, Stamps et al. 1987, Bell 1991, Goodwin and Fahrig 2002, Conradt et al. 2003, Heinz and Strand 2006). Animals searching for suitable habitat on a landscape are expected to experience an elevated risk of mortality and, according to foraging theory, should maximize efficiency by adopting the best search strategy in response to the scale of patchiness (Stephens and Krebs 1986, Zollner and Lima 1999). However, at present, there is limited information concerning the scales at which insects perceive habitat patchiness and how this perception influences decisions regarding movement and selection of reproductive sites. Grasshoppers have been shown to respond the spatial pattern of habitat at different scales while moving on heterogeneous landscapes (With 1994a). A highly vagile, large grasshopper, *Xanthippus corallipes*, with affinities for two common habitat types were able to aggregate in response to their preferred habitats. The population occupied 22% of the landscape with most individuals concentrated in only 2% of their territory. In contrast, a small grasshopper, *Psoloessa delicatula*, with low vagility and an affinity for rare habitat were not able to aggregate in their preferred habitat. This small grasshopper was found in 67% of the landscapes and most individuals were spread over 30% of the landscape. A study of movement of adults and nymphs of a grasshopper, *Opeia obscura,* showed that the two developmental stages were responding to landscape structure in different ways. Differences in size and the activities in which the two stages were engaged may have impacted their perception of the scale of heterogeneity and, thus affected their interactions with landscape structure (With 1994b). These examples illustrate how movement behavior of individuals may result in patterns of resource use that do not coincide with the pattern of preferred habitat in a landscape. Rather than strictly following habitat distribution, patterns of resource use may be strongly influenced by the scale at which a species or an individual perceives habitat pattern. How an insect perceives and responds to the pattern may depend on behavioral activity in which it is engaged (i.e. searching, feeding, ovipositing) (McIntyre and Wiens 1999b), developmental stage and constraints on movement ability (With 1994b), and perceptual range (Romero 2007a).

In the present study, two key ecological processes - movement and oviposition - are studied in relation to the patchiness of habitat on experimental landscapes using red flour beetle (*Tribolium castaneum*) as a model. I propose that if beetles do not respond to the spatial pattern of habitat, then the distributions of movement pathways and oviposition sites will be coincident with that of habitat. Alternatively, I predict that behavior of beetles will be modified by habitat pattern so that distribution of movement and oviposition will not be coincident with that of the habitat or, perhaps, even each other. Landscape structure may affect the functions of movement and oviposition differently. To assess how habitat fragmentation affects movement and oviposition patterns I tested red flour beetles in landscapes created with the same amount of resource, but with three levels of habitat fragmentation: 1) clumped, 2) intermediately fragmented, and 3) highly fragmented. By comparing distributions of movement and oviposition sites in relation to habitat fragmentation, I may be able to elucidate behavioral mechanisms that vary with both beetle activity and landscape structure. To accomplish my objectives I examined how various factors affected distribution of movement and oviposition sites including: 1) patch size, and 2) number of edges, and 3) time.

Separate experiments were conducted to provide supplemental information that could help interpret findings of the landscape study. In the main landscape study I was able to record the location of beetle movement on a daily basis but could not determine if locations were revisited within or between days or when eggs were laid. To determine if beetles revisited locations over time I video-recorded movement on the various landscapes over a 48 h period in a separate experiment. In preliminary studies I observed that females often did not oviposit for more than 24 h after being removed from an infested patch (i.e., rearing colony) and placed into a new resource. To pinpoint more closely when eggs were laid on the landscapes I conducted a separate study to document how transfer from the colony to new resource affected the amount and timing of oviposition. And finally, I assessed the potential fitness consequences of a female's oviposition decision in relation to habitat fragmentation in an experiment documenting the survival of beetles to adulthood. Treatments in this experiment attempted to mimic patches on the landscapes and, thus, consisted of patches with different spatial distributions, amounts of resource, and egg densities.

Red flour beetle is well suited to empirical studies concerning the influence of landscape structure on movement and oviposition because the anthropogenic environments in which these insects thrive are patchy landscapes where they find and exploit small flour patches (Campbell and Hagstrum 2002, Campbell and Runnion 2003). The dispersal behavior of this species has also been extensively studied (Naylor 1961, Hagstrum and Leach 1972) although not typically in the context of landscape structure. In an earlier study, I showed that red flour beetles modified search strategy in response to variation in inter-patch distances and amount of habitat edge produced by habitat fragmentation. Landscape structure altered movement parameters such as path tortuosity, displacement, and time spent on the landscape as well as in individual patches (Romero 2007b). If red flour beetles respond to differences in landscape structure by changing their search strategy, then perhaps other important ecological functions, such as oviposition, may be affected as well.

Methods

Habitat fragmentation and resource use

Female beetles used in this study were taken from a lab colony founded from individuals collected from a flour mill ~ 34 months before experiments were conducted. Sub-colonies from which beetles were removed had been initiated with ~ 75 adult beetles (mixed sex) and maintained in 0.25 L of wheat/brewers yeast mixture (95/5%) under the same environmental conditions. Age of the experimental beetles was standardized to 3 weeks ± 4 days by sub-culturing colonies every 3 or 4 days and then removing the initial adults after they had been in the new culture for 4 days. Beetles used in experiments were removed randomly from sub-colony jars just prior to use. Females were identified by observing with a microscope the absence of the setaceous patch present on the first femur of males (Good 1936). The experiment was conducted in a walk-in environmental chamber (27 ± 0.03° C; 56 ± 5% rh; 14:10 day/night cycle).

To assess how landscape structure may influence movement and oviposition, the degree of fragmentation was manipulated in three experimental landscapes and the number and position of cells visited and eggs deposited by females, over a 48-h time period were recorded. Three fractal landscape treatments were created, each with a different level of habitat fragmentation, using RULE, a freely available software program (Gardner 1999). Values of H (a parameter that controls the degree of spatial autocorrelation among habitat cells on the landscape) input to the program were 0.2, 0.5, and 0.9, producing a highly fragmented (fragmented), an intermediately fragmented (intermediate), and a highly clumped landscape (clumped), respectively. The RULE software outputs binary maps with habitat designated as 1 and non-habitat, or matrix, designated as 0 on a regular grid. On landscapes, habitat cells were comprised of wheat flour and matrix cells did not contain flour. The extent of each landscape was 64 x 64 cm with a grain size of 2 x 2 cm (i.e., habitat cell) in 32 rows and columns. I chose the 2 x 2 cm grain size because it was the smallest grain size practical in creating my landscapes. Creation of fractal landscapes using RULE requires the use of a parameter "L" that controls the number of iterations of the midpoint displacement algorithm that creates the fractal pattern. The extent of fractal landscapes are always equal to 2^L (i.e. when L = 4 the dimension (rows and columns) of the landscape map = 16). I set "L" at 5 so that my landscapes would measure 64 x 64 cm which was the largest extent practical for my experimental design. Abundance was set at 10% with each landscape having 108 habitat cells. I set habitat abundance at 10% because previous studies showed that the effects of habitat fragmentation on movement of Tenebrionid beetles in experimental fractal landscape were greater when habitat abundance was lower than 20% (Wiens et al. 1997). Although habitat abundance was held constant, habitat cells were arranged in different patterns according to the fragmentation treatment. The difference in fragmentation level caused each landscape to have different numbers and sizes of habitat patches (i.e., contiguous groups of habitat cells with shared edges and corners) (Fig. 1).

The binary map produced by the computer program was copied onto a piece of heavy cardstock marked with a grid containing 32 rows and columns for a total of 1024, 2 x 2 cm cells. Cells designated as habitat were then cut out creating one template for each landscape fragmentation treatment. Templates were placed onto new white paper covering a table within an environmental chamber and a layer of flour applied over the template and the template then removed to create the patterns of flour patches. On each landscape, there was approximately 22 g of flour (2.4 g per habitat cell) applied to a depth of approximately 3 mm. This flour depth was deep enough to cover beetles as they tunneled into the flour. To confine beetles on the landscape, a 92-cm diameter circle of plastic tubing (I.D. 0.75 cm) filled with sand and with the ends connected with a wood dowel was placed around each landscape. Red flour beetles rarely fly, are poor climbers, and mainly disperse by walking so the plastic tubing was sufficient, in most cases, to confine them in the landscapes.

One female beetle was released into the matrix approximately 6 cm from the plastic ring at the top of each of the three landscapes after acclimation under an inverted 14.79-ml glass vial for 3 minutes. Beetles were then allowed to explore the landscape for 48 hours. Because beetles exploring habitat tunnel into the flour, leaving evidence of their movements, I was able to record the number and location of habitat cells first visited by beetles between 0-24 and between 24-48 hours by checking each flour cell for disturbance. After 48 hours, each habitat cell was removed individually from the landscape, the flour passed through a U.S. size 50, 0.353 mm sieve, and the location of each habitat cell and the number of eggs it contained recorded. One replicate of each of the three fragmentation treatments was run simultaneously as a block in time and replicated 16 times sequentially over the course of 8 weeks.

I calculated lacunarity indices (Plotnick et al. 1993) of habitat pattern for the three fragmentation treatments, as well as lacunarity index values for the cells through which beetles moved (visited cells), and cells with eggs (oviposition sites) in all landscapes. The term lacunarity describes landscape texture, or the variability of gap sizes among locations. The lacunarity index (Mandelbrot 1983, Plotnick et al. 1993) is a metric useful for comparing the distributions of different spatial locations in the same or in different landscapes (McIntyre and Wiens 2000). McIntyre and Wiens compared lacunarity index values between habitat and movement patterns (landscape use) to determine if Tenebrionid beetles responded to the scale and pattern of habitat patches. They hypothesized that if beetles responded to the spatial pattern of patches, then their response would either follow habitat pattern linearly (patches used according to size or proportion) or follow in a non-linear manner (use not according to size or proportion). McIntyre and Wiens used a two-step process in their study. First they compared lacunarity index values of habitat and movement pattern to determine if beetles were sensitive to the spatial pattern of habitat, then to elucidate how habitat pattern influenced function (movement) they plotted natural log-transformed lacunarity values of habitat pattern versus natural log-transformed values of the movement pattern and determined if the resulting curve departed from linearity. In this study lacunarity was used to quantify and compare the variation in gap size among habitat cells, cells through which beetles moved, and cells with eggs.

To calculate lacunarity, a box (window) representing a specific scale of measurement (e.g., 10 x 10 cm) is systematically moved across the rows and columns of a regular grid on which is mapped binary (presence–absence, 0-1) data of a spatial pattern. Gaps in the pattern are measured using a moving window algorithm which calculates mean and variance values of the scores of 0's within each box, converting them into an index which is a ratio of the calculated mean and variance values. For example, in the case of landscape pattern, a high lacunarity value indicates more variation and larger gaps within the spatial pattern of habitat cells, whereas a low lacunarity value results when habitat cells are more evenly dispersed and gaps are smaller and less variable. For movement pathways and oviposition sites, a high lacunarity value indicates that cells through which beetles have moved or in which they have placed eggs are restricted to a localized area (aggregated) and there are large or irregular gaps in the pattern. A low lacunarity value occurs when cells through which beetles have moved or have placed eggs are scattered over a wider area of the landscape and gaps are smaller and more regularly spaced.

I used ANOVA (GLM procedure, SAS Institute, Inc., 2002) and Tukey's HSD to test for differences in lacunarity among the three habitat fragmentation treatments. Within each fragmentation treatment I compared lacunarity of habitat pattern, visited cells, and oviposition sites. To investigate the relationships of the spatial distributions, I plotted natural log-transformed lacunarity values of habitat pattern and visited cells versus those of oviposition sites. I used Tablecurve2D software (Systat Software, Inc. 2006) to fit regressions to a line of best fit and determine departure from linearity.

An analysis of variance (ANOVA, mixed model procedure) and Tukey's HSD for means separation were used to test the main effect of fragmentation treatment on the following response variables: total number of habitat cells visited, total number of eggs on landscape, total number of habitat cells with eggs, and total number of eggs in cells with eggs. Blocks were modeled as a random effect in the mixed model. Comparisons were made between cells initially visited from 0-24 h (day 1) to those first visited from 24–48 h (day 2) for the following variables: number of new habitat cells visited, number of cells with eggs, and number of eggs per cell with eggs. To test for differences in the number of visited cells between days, Chi-square analysis of association based on Pearson's exact test (SAS Institute, Inc. 2002) was used to determine if equal proportions of new cells were visited on the first and second day.

I investigated how patch size influenced both the number of visited habitat cells and eggs in the highly fragmented and intermediately fragmented landscape. I did not investigate the role of patch size in the clumped landscape because it was composed of one very large (105 cells) and one very small patch (3 cells). I placed patches (a single cell or a group of cells, contiguous at edges and corners, that were completely surrounded by matrix) into 4 size categories (1 cell, 2-4 cells, 5-9 cells, and 10-17 cells) and added an additional category (> 17 cells) for the intermediate because one large patch in this landscape was composed of 63 cells. These categories were chosen to provide the widest range of patch sizes for a practical comparison. Chi -square analysis of association (Mantel – Haenszel exact test, SAS Institute, Inc., 2002) was used to test if the number of eggs expected to be present in a habitat patch of a specific size (based on proportion of the landscape made up of patches of that size category) was similar to the observed number of eggs. Chi-square analysis of association (Mantel – Haenszel exact test) was also used to test if the number of habitat cells expected to be visited (based on proportion of the landscape made up of patches in a specific size category) was similar to the observed number of habitat cells visited.

The role of edges in beetles' choice of oviposition site on the clumped landscape was investigated. I did not examine this relationship in the fragmented or the intermediate landscape because few cells were without an edge. I compared observed versus expected numbers of cells with eggs that had either no matrix cell adjacent or at least one matrix cell adjacent (Pearson's exact test, alpha = 0.05). The number of expected cells was based on the proportion of cells in the landscapes with either no adjacent matrix cell (40%) or with at least one matrix cell adjacent (60%).

The design of the main landscape experiment prevented movement to be video recorded at the time the experiment was being conducted. To determine if beetles revisited cells on the landscapes I video recorded movement on the three landscapes over a 48-hr observation period separately from the main experiment. Parameters measured included number of new cell visits, number of cells revisited on the same day, and number of cells revisited on day two which were initially visited on day one. I video recorded the movement of one beetle over a 48-h period in one of the three differently patterned landscapes using three video cameras (each recording an overlapping portion of the landscape) suspended over the arena and connected to a multi-channel digital video recorder. Two reflective lighting fixtures with 25 watt red incandescent bulbs were suspended over the arena, providing light for night recording. These lights were left on continuously during the duration of the trial. I repeated this procedure 3 times for each landscape (n = 3 per each fragmentation treatment). Trials were conducted under ambient conditions ($19.44 \pm 0.74^{\circ}$C; L:D - 13:11 h). Video recordings were reviewed and the location of the beetle (either in a specific habitat cell or in the matrix) and number of newly disturbed habitat cells were recorded at hourly intervals. The number of cells revisited was quantified by reviewing the recording for a period of 10 minutes before and after each hourly time point (for a total of 20 min per hour) and recording all cell locations visited by the beetle during that time period. At some time points, I could not determine the exact location of the beetle. In these cases, I assumed the beetle was inactive and hidden under the flour and so recorded the beetle as being in habitat with exact location unknown.

To help evaluate the amount and temporal pattern of oviposition during the main landscape experiment I conducted a separate oviposition experiment. In this experiment I quantified the number of eggs laid every 24 h in three different treatments. I transferred beetles from colony jars (as performed at the start of the landscape study) into the following treatments: 1) previously infested or conditioned flour, 2) fresh uninfested flour, and 3) no flour for a 24-h period followed by fresh flour on subsequent days. Flour conditioning has been shown to affect dispersal and suppress oviposition in red flour beetles (Ghent 1963, Sokoloff 1974). Conditioning results from 1) depletion of nutritive content, 2) accumulation of feces, exuviae, and dead imagoes, and 3) defensive compounds, such as quinones produced by beetles as the colony grows in a limited resource. Because a female was removed from a colony with conditioned flour and then placed on landscapes with fresh flour I wanted to examine how removal from conspecifics and conditioned flour affected oviposition. Beetles used in the experiment were taken from a sub-colony jar containing 115.2 g of conditioned flour and ~ 2,085 adult beetles aged 3 weeks ± 4 days (normal conditions for sub-colonies). Eggs and larvae were sieved from the flour and the remaining flour used as the source of conditioned flour for the experiment. After beetles were counted and conditioned flour weighed, a single female beetle was placed immediately into a 2 oz. covered portion cup containing 2.0 g of either fresh flour, or conditioned flour. Females were subsequently transferred each day into a new container containing the appropriate flour treatment. A third treatment allowed me to assess the impact of the lack of habitat (and/or food) on oviposition. For this treatment I placed a single female into an empty cup for an initial 24-h period and subsequently transferred her into fresh flour each day for the remainder of the experiment. Each treatment was replicated four times in each of three blocks for a total of 12 replicates per treatment. Four trays, with each tray containing one replicate of each treatment, were placed on three different shelves within an environmental chamber. Temperature was set at 28 ± 0.53° C and lighting set to a 14:10 day/night cycle. After each female was transferred into a new portion cup each day, flour from the previous day was sieved and eggs were counted. Analysis of variance (ANOVA), repeated measures, and Tukey's HSD for means separation were used to test main effects of flour treatment and hour after removal from colony on number of eggs. Blocks were modeled as random effects.

Effects of resource amount, fragmentation, and egg density on progeny fitness

To determine impact of the amount and spatial pattern of resource on the fitness consequences (i.e., survival to adult and adult size) of an oviposition decision (i.e., number of eggs laid in a cell), I performed the following experiment. There were five patch fragmentation treatments consisting of a single habitat cell (2 x 2 cm) with: 1) no other habitat cells around it (0.04 g total flour available), 2) one adjacent habitat cell (0.08 g total for all cells), 3) two adjacent cells (0.12 g total), 4) one additional habitat cell 15 cm away (0.08 g total), and 5) two additional habitat cells each 15 cm away (maximum distance was constrained by arena size) from the egg cell (0.12 g total). Each of the five treatments was combined with one of three egg densities (6, 12 and 18), resulting in 15 different treatments combinations. Cell size was based on the size of a single cell of my main landscape study (2 x 2 cm) and resource amount and egg densities were selected based on Campbell and Runnion (2003).

Each treatment combination was placed in a 22.5 cm^3 square plastic uncovered container (The Cary Co., Addison, IL U.S.A.) which served as an escape-proof experimental arena. The bottom of the container was spray-painted with flat gray automotive primer (Rust-Oleum Inc.). Habitat cells were created as described above and all cells were $\geq$ 2 cm from the edge of the container to lessen influence of edges on larval movement. Immediately prior to setting-up treatments within arenas, I obtained eggs from females that had been placed in fresh flour for a 24-hr period. Eggs were sieved from flour as described above and immediately added to a single flour patch in each treatment combination. Three single patch treatments and two fragmentation treatments combined with three egg densities were placed into blocks of fifteen treatment combinations. Blocking was achieved by initiating replicates of the fifteen treatments on different days. Blocks were replicated 5 times over the period of one week, resulting in 5 replicates of each treatment combination.

I placed experimental arenas on shelves within a controlled-environment walk-in chamber. Shelf assignment for blocks, treatment position on shelves, and position of the patch receiving eggs were randomized. Treatments were incubated for 35 days at 28 ± 0.03˚ C, which is adequate time for eggs to reach maturity (Sokoloff 1974). A 14:10 h, light/dark cycle was maintained for the course of the experiment. At the end of the experimental period, I removed and counted all surviving adults, pupae, and larvae from the arenas. Adults were sacrificed by freezing for 20 min, adhered to a paper card, and photographed with a digital camera. Elytral length was measured from digital images using Scion software (Scion Corp. 2005) and the average length of the two elytra was used as a measure of adult size for analysis. Analysis of variance (mixed model procedure) was used to test main effects of resource amount, fragmentation, and egg density on proportion of all life stages (adult, pupa, and larva), as well as proportion of adults surviving 35 days post-oviposition. Blocks were modeled as random effects. Proportions were arcsine square root transformed before analysis to normalize data (Zar 1999).

Results

Habitat fragmentation and resource use

The degree of fragmentation imposed by my treatments resulted in different numbers and sizes of flour patches, amount of habitat edge, and average distance between patches (Figs. 1 & 2). There were 36 patches on the fragmented, 13 on the intermediate, and two on the clumped landscape. For the both the fragmented and intermediate landscapes the distribution of patch sizes was skewed to the left because there were many more small patches (1-10 cells) than larger patches. The largest patch on the fragmented landscapes was composed of 12 cells, but the largest on the intermediate landscapes had 63 cells. The large patch on the clumped landscapes had 105 cells and the small had 3 cells. The fragmented landscape had the highest amount of edge, or total linear measure in meters of edges of habitat cells adjacent to a matrix cell, at 5.88 m. The intermediate landscape had 3.9 m of edge and the clumped had the least, 1.5 m. The average distance between habitat cells was 5, 4, and 3 cm for the fragmented, intermediate, and clumped landscapes, respectively.

Based on lacunarity analyses, beetle response to habitat pattern differed depending on the activity in which they were engaged. Higher lacunarity values indicate that distribution of oviposition sites (cells with eggs) was significantly more aggregated than distribution of visited cells and habitat cells ($F = 18.88$, $df = 2,135$; $p < 0.0001$) while the distribution of visited cells was similar to that of the habitat ($p = 0.3971$) (Fig. 3). Lacunarity of fragmented habitat was significantly lower than in intermediate and clumped landscapes ($F = 8.92$; $df = 2,45$; $p = 0.0005$) (Fig. 3), indicating a more dispersed distribution of habitat cells. However, lacunarity of visited cells (F = 0.89; df = 2,45; p = 0.4190) and oviposition sites (F = 0.34; df = 2,45; p = 0.7110) showed no significant differences among landscape fragmentation treatments.

Differences in lacunarity values between habitat pattern and oviposition sites and visited cells and oviposition sites suggest that oviposition site choice was influenced by both the underlying habitat pattern and the pattern of visited cells. Because there were differences in these lacunarity values I plotted the natural log-transformed lacunarity values of habitat cells and visited cells versus oviposition site lacunarity to explore their relationships. Resulting curves for the fragmented and intermediate landscapes were best fit by power functions, while the curves for the clumped landscape were described best by exponential functions (Fig. 4; Table 1). These curves demonstrate that the distribution of oviposition sites to those of habitat or visited cells had relationships that could not be explained by simple linear functions. Ninety-five percent confidence intervals for the curves resulting from plotting visited cell lacunarity versus oviposition site lacunarity for the different landscapes did not overlap (Fig. 4A; Table 1). The shapes of the curves for the various landscapes were slightly different, but close together, and there was relatively little change in their relative positions as scale increased. This indicates that the relationship of the distribution of oviposition sites versus visited cells differed somewhat among fragmentation treatments, but the differences remained relatively constant with increasing scale. Ninety-five percent confidence intervals for the curves resulting from plotting habitat cell and oviposition site lacunarity also did not overlap (Figure 4B; Table 1). In this case, the shapes of the curves were very different from one another, further apart, and their relative positions changed over scales of measurement. These observations suggest that the relationships of the distribution of oviposition sites versus habitat patterns were distinctly different among fragmentation treatments, more complex, and varied over scale.

At the extent of the entire landscape, there were no differences in number of habitat cells visited ($F = 1.07$; df = 2, 75; $p = 0.3488$) (Fig. 5A) nor in total number of eggs ($F = 1.90$; df = 2, 30; $p = 0.1664$) among the three fragmentation treatments (Fig. 5B). However, beetles visited more than twice the number of cells during the first 24 h than during the second 24-h period ($F = 136.44$; df =1, 75; $p < 0.0001$) among all fragmentation treatments (Fig. 5A). There were no interactions between landscape fragmentation treatments and day ($F = 1.59$; df = 2, 75; $p = 0.2106$) for number of new cells visited. Chi-square analysis of association of observed versus predicted number of new cell visits showed that the number of new cell visits observed for day two was significantly less than predicted based on the proportion of cells visited on day one, for all landscape treatments (Pearson Chi-square exact test - fragmented, $\chi^2 = 6.2017$; df = 1; $p = 0.0212$; intermediate, $\chi^2 = 11.1429$; df = 1; $p = 0.0014$; clumped, $\chi^2 = 11.1429$; df = 1; $p = 0.0014$) (data not shown).

Although the total number of eggs was not different among the landscapes, there were differences in the spatial arrangement of cells with eggs and the number of eggs per cell depending on the level of habitat fragmentation. There were significantly more cells with eggs in the clumped than in the fragmented or intermediate landscape ($F =6.89$; df =2,75; $p = 0.0018$) (Fig 6A). The interaction effect was significant between fragmentation treatment and day of initial visit for number of cells with eggs ($F = 3.94$; df = 2,75; $p = 0.0237$. In the intermediate and clumped landscapes there were significantly more cells with eggs among cells initially visited on day one than on day two (intermediate - $p < 0.0001$; clumped – $p < 0.0001$). However in fragmented landscapes the number of cells with eggs was similar between days ($p = 0.1947$). Among fragmentation treatments there were significantly fewer cells with eggs among cells initially visited on day 1 in the fragmented landscape than the clumped landscape ($p = 0.0005$) and the number in the intermediate landscape was intermediate and not significantly different from the other two fragmentation treatments (fragmented – $p = 0.3848$; clumped – $p = 0.1598$). On day two there were no significant differences in the number of cells with eggs among fragmentation treatments (fragmented – intermediate, $p = 0.9255$; fragmented – clumped, $p = 0.9919$; intermediate – clumped, $p = 0.6307$).

The number of eggs per cell with eggs was significantly less in clumped landscapes than on fragmented landscapes and the number of eggs per cell with eggs on intermediate landscapes was intermediate and not significantly different from the other two fragmentation levels ($F = 5.12$; df = 2, 75; $p = 0.0082$) (Fig. 6B). There was no statistical difference between days for number of eggs per cell within and among all fragmentation treatments ($F = 3.88$; df = 1,75; $p = 0.0527$). There was also no fragmentation treatment and day of initial visit interaction ($F = 0.04$; df = 2,75; $p = 0.9582$)

Number of visits generally increased with patch size (group of habitat cells) in both fragmented and intermediate landscapes, with the observed number of habitat cells visited not significantly different from expected based on patch size for the fragmented landscape (Mantel – Haenszel exact test; $\chi^2 = 2.439$, df = 1, $p = 0.167$) (Fig. 7A), but different from expected based on patch size for the intermediate landscape (Mantel –Haenszel exact test; $\chi^2 = 3.676$, df = 1, $p = 0.0083$) (Fig. 7B). In the intermediate landscape, the number of observed visits to smaller patches (1 cell and 2-4 cell patches) closely matched the expected number based on the proportion of the landscape occupied by patches of that size. In contrast, the number of observed visits to patches with 5-9 and 10-17 cells was higher than predicted, while the observed number of visits to the largest patch (> 17 cells) were less than predicted based on the proportion of the landscape occupied by patches of that size.

Results for number of eggs showed a similar pattern to number of visits; no significant differences between observed and expected on the fragmented landscape (Mantel –Haenszel exact test; $\chi^2 = 2.559$ df = 1, p = 0.0844) (Fig. 7C), but significantly different on the intermediate landscape (Mantel –Haenszel exact test; $\chi^2 = 2.707$, df = 1, p = 0.0083 (Fig. 7D). In contrast to results for visited cells in intermediate landscapes, the number of observed cells with eggs in smaller patches (with 1 cell and 2-4 cells), was less than expected based on the proportion of the landscape occupied by patches of that size. The number of observed cells with eggs in patches with 5-9 and 10-17 cells was higher than predicted, while the observed number of cells with eggs in the largest patch (> 17 cells) was less than predicted. Beetles appeared to favor movement in habitat cells with an edge adjacent to the matrix rather than movement in interior cells in the clumped landscape ($\chi^2 = 6.474$, df = 1, p = 0.0013) (Fig. 8A). This was not the case for oviposition sites (cells with eggs) because beetles oviposited in cells with at least one edge and with no edge in approximately equal proportions ($\chi^2 = 1.539$, df = 1, p = 0.264) (Fig. 8B).

Video recording of beetles documented that interactions with habitat varied widely among individuals and over time, but on fragmented and intermediate landscapes, generally more cells were visited on the first day compared to the second day (Fig. 9). Some beetles entered habitat patches within a few minutes of release, remaining relatively inactive; others moved in, out, and around edges of habitat patches, moving at a relatively fast rate before eventually entering habitat patches. The supplemental video tracking data allowed us to verify that beetles do revisit previously visited habitat cells, on both the first and the second day. During video tracking it was possible to observe more visits to cells than in the previous experiment. This difference can be explained because direct observation of the video allowed even brief visits, which left no visible sign of disturbance, to be recorded. Whereas, in the previous experiment, these brief visits were not detectable due to using visible disturbance of the flour as the only indication of cell visitation. Comparison of proportions of cells with visible disturbance between the two experiments showed that the degree of visitation based on visible disturbance to cells was relatively similar (data not shown).

Results of the separate oviposition experiment showed that, after removal from the colony, beetles produced few eggs in treatments with new flour in the first 24 h, but oviposition significantly increased on subsequent days. There were significant treatment and interaction effects (flour effect, $F = 8.37$, $df = 2,158$, $p = 0.0004$; day effect, $F = 14.71$, $df = 4,158$, $p < 0.0001$; and flour-day interaction, $F = 5.66$, $df = 8,158$, $p < 0.0001$) (Fig. 10). In the conditioned flour treatment, oviposition was not significantly lower during the first 48 h of the experiment than during subsequent days (Fig. 10). Oviposition by beetles in conditioned flour was lower than that of beetles immediately transferred to new flour at all time points. For beetles transferred to new flour, oviposition was lower for the first 24 h in new flour, whether transfer was direct to the flour from the colony jar or if the beetle had been held for 24 h without food. Beetles held without flour (starved) for 24 h did not oviposit during that initial period without food and during the first 24 h in new flour the amount of oviposition was not different from beetles held in conditioned flour. However, after the first 24 h in new flour (48 h after removal from the colony), oviposition did not differ from beetles immediately transferred to new flour and was greater than beetles in conditioned flour.

Effects of resource amount, fragmentation, and egg density on progeny fitness

Amount of resource and fragmentation had no impact on total number of individuals that survived (all life stages combined) (Fig. 11A), but did significantly increase survival to adulthood in the treatment with 6 eggs and one additional habitat cell (Fig. 11B). Although there was no effect of resource amount/fragmentation treatments ($F = 1.25$; $df = 4,56$; $p = 0.3016$), egg density did have an overall effect on survival of all life stages ($F = 11.14$; $df = 2,56$; $p < 0.0001$) to 35 days post-oviposition. Significantly more individuals were present in the 6 egg treatments compared to the 12 and 18 egg treatments (Fig. 11A). There was no interaction effect between resource amount/fragmentation and egg density treatments (F = 0.33; df = 8,56; p = 0.9524) on survival of all life stages. Compared to survival of all life stages, where resource amount/fragmentation treatments had no effect on survival at any egg density, the ability of individuals to reach adulthood was significantly increased in the six-egg treatment by both resource amount/fragmentation (F = 7.55; df = 4,56; p <0.0001) and egg density (F = 16.99; df = 2,56; p <0.0001) (Fig. 11B). There were no significant interactions between resource amount/fragmentation and egg density treatments (F = 1.35; df = 8,56; p = 0.2372) on survival to adulthood. When results for the single cell treatment were compared to those treatments with two or three adjacent cells, there was no significant difference in survival to adult, nor any difference due to density of individuals. When the single cell treatment was compared to the treatment with more resource that was divided into 2 additional cells (fragmented), significantly more individuals survived to adult when there were not more than 6 eggs. Comparisons between the single cell treatment and the 3 additional cell treatment revealed no significant differences in survival at any egg density. Comparisons between treatments with comparable amounts of resource (i.e. 1 adjacent with 1 additional and 2 adjacent with 2 additional) revealed no significant difference in survival to adult. No difference in adult size based on elytra length among treatments was found (Table 2). During the course of the experiment, it was observed that all flour patch cells were visited by larvae; disturbance to flour was evident just days after expected egg hatch.

Discussion

Female red flour beetles respond to landscape structure differently depending on the activity in which they are engaged. In my study, the distribution of visited cells was similar to that of the underlying habitat pattern, indicating that, when moving on a landscape, beetles were not sensitive to patchiness. Given that beetles had 48 h to explore, it is not surprising that they could access all areas of my relatively small experimental landscapes. Theory states that vagile species may not perceive patchiness at relatively small spatial or temporal scales during dispersal (Kotliar and Wiens 1990, With 1994a). In contrast to movement, when beetles were choosing oviposition sites, they apparently were sensitive to the pattern and scale of patchiness because the distribution of oviposition sites was not consistent with and was more aggregated than the underlying habitat pattern. It appears that, during oviposition, beetles perceive patchiness at a finer scale than when moving in the landscape. Beetles were also sensitive to the pattern of previously visited cells when choosing oviposition sites. Therefore, for red flour beetles, selection of oviposition sites appears to be a complex process related to the underlying pattern of habitat as well as to the pattern of previously visited locations.

Regression curve analyses of distributions of habitat and visited cells versus oviposition sites give support for the existence of perceptual and functional differences among landscape fragmentation treatments. Differences in the shapes of the curves show that beetles did not respond to habitat pattern and previously visited cells in the same way during the oviposition process. Curves for visited cells vs. oviposition sites are more consistent in shape and relative position among fragmentation levels and less steep than curves for habitat pattern vs. oviposition sites. This means that, while ovipositing, beetles followed the distribution of visited cells in a similar manner across levels of habitat fragmentation and scales, but responded to the distribution of the underlying habitat pattern in a more complex manner and at finer scales. It is interesting to note that curves for fragmented landscapes of both relationships have the highest lacunarity values, indicating higher aggregation of oviposition sites relative to the intermediate and clumped landscapes.

How beetles perceived the landscape changed with beetles' activities and these activities changed over time. Female beetles appeared to be engaged in exploratory behavior when first released onto the landscape. Beetles visited more new cells in the first 24 h than in the second 24-h period. Delay in oviposition until the second day and revisitation of previously visited cells suggests that females may have returned to certain cells or patches to oviposit on the second day. It is likely that release from the suppressive effects of the conditioned flour in which the beetles were reared may have elicited a dispersive exploratory phase (Ziegler 1976) during which beetles evaluated habitat. After the initial exploratory period, the presence of fresh flour resource may have stimulated oviposition or the suppressive effect of the conditioned flour dissipated and oviposition resumed at a higher rate. Conditioned flour can inhibit oviposition in flour beetles (Prescott 1970), up to 78% depending on the amount of conditioning (Sokoloff 1974a, Sonleitner and Guthrie 1991). It has been reported that female flour beetles, when placed into even slightly conditioned flour, immediately decrease oviposition and that this inhibition is immediately or quickly reversed when females are placed back into fresh flour (Sokoloff 1974).

When beetles were engaged in exploratory behavior on the first day, patchiness may have been less important than when beetles were engaged in oviposition on the second day. There were no differences in beetle movement parameters among landscape fragmentation levels such as the overall number of habitat cells visited, or in the total number of eggs laid; however, there were differences in the number of habitat cells containing eggs and the number of eggs per habitat cell. At the broadest scale examined, the extent of the landscape, beetles visited similar numbers of habitat cells and laid similar numbers of eggs among all fragmentation treatments, indicating that they encountered enough resource to support the total number of eggs laid or their perception of the total amount of resource was similar. More likely the amount of oviposition was constrained by the number of eggs available during the 48 h period. However, at the scale of individual cells, females placed fewer eggs in more individual habitat cells in clumped landscapes than in intermediate and fragmented landscapes.

Why would females spread eggs in a large amount of resource and aggregate eggs in a small amount of resource? If beetles perceive fine scale differences in patchiness when ovipositing, then when habitat is more fragmented, they may lay more eggs per cell than in the clumped landscapes, because they perceive there is less resource available or that finding large patches is more difficult. Oviposition sites were proportional to the number of cells visited on a particular day, with more oviposition sites chosen from cells initially visited on day one in intermediate and clumped landscapes. However, in fragmented landscapes, beetles did not preferentially choose to oviposit in cells visited initially on day one, instead allocating eggs more equally among sites visited on either day. Taken together, these observations suggest that beetles made fewer cell transitions in fragmented landscapes than on clumped landscapes during the second day when oviposition was taking place. Perhaps experience on the fragmented landscape during the exploratory period changed a female's oviposition strategy and locations for eggs were chosen more randomly than on the other landscapes, or beetles perceived travel as more risky causing them to move less between cells, preventing them from finding and ovipositing preferentially in cells previously visited. Transitions from cell to cell on clumped landscapes may have been perceived by beetles as easier and less risky because they did not need to travel in the matrix in order to maximize the spread of eggs.

Mechanistically, staying longer and ovipositing more in small, isolated patches may be a function of the beetles' perception of the edges of the cell and how they respond on encountering these edges (Romero 2007a, Romero 2007b). If the cell edge is a habitat-matrix edge, beetles may turn back and thus spend more time in the individual cell and, therefore concentrate more eggs there. I can only speculate on what mechanisms may drive aggregation of eggs in fragmented landscapes; however, on my clumped landscapes, data shows beetles clearly preferred to move in habitat cells with an edge on the matrix, but when ovipositing this preference declined and cells with eggs were evenly distributed among edge cells and interior cells. Beetles may have moved into interior cells in clumped landscapes during oviposition in an attempt to further spread or protect eggs.

Beetles exhibited a preference for visiting and placing eggs in larger patches on both the fragmented and intermediate landscapes. These results are indicative of the complexity of beetles' interactions with the patchiness of the landscapes; in fragmented landscapes the response to patch size is more linear, but on intermediate landscapes the response is more complex. Findings of the present study, indicating preference for oviposition in larger resource patches, agree with results of a previous study on patch exploitation by red flour beetles (Campbell and Runnion 2003). In that study females, when presented with a choice, laid more eggs in larger patches, adjusting clutch size in order to maximize fitness gains. In the same study, when given four choices among equal sized patches, females distributed eggs equally among all the patches. Thus, the previous report coincides with the present study, showing that beetles laid equal numbers of eggs in landscapes containing equal amounts of resource. That females tend to oviposit in larger patches, providing more resource to developing progeny, while at the same time attempting to spread eggs, as in the clumped landscape, suggests that females are making oviposition choices possibly to avoid cannibalism or some other detrimental effect of crowding on progeny. Flour beetles are well known for their cannibalistic behavior toward eggs and pupae (Stevens 1989) although crowding per se has been reported to have no adverse effects on development when resources are adequate (Park 1938).

It has been noted that selection on oviposition choice should be weak for female insects that are generalist feeders or that have grazing larvae because larvae can leave a poor oviposition site to find better resources (Thompson 1988). Red flour beetles are generalist feeders and larvae are able to disperse away from oviposition sites. Thus, females should not, as predicted by Thompson's model, be selective about oviposition site choice. In this study, however, behavior of females did not support Thompson's prediction as they did show evidence of oviposition site choice. Moreover, the resource amount/fragmentation study documents that individuals reach adult stage more quickly when there is more resource. Therefore, my results indicate that females are choosy about oviposition sites and their choices have consequences for relative fitness.

In contrast to the conclusion reached for oviposition choice on intermediate and clumped landscapes, it appears that some characteristic of the fragmented landscape caused females to aggregate eggs in fewer locations and to use small patches even when larger patches were available. As mentioned earlier, if females perceived travel costs to be too high in fragmented landscapes, then this could explain their behavior. Aggregated oviposition in the fragmented landscapes may be explained by the evolutionary stable strategy theory of parent-offspring conflict in herbivorous insects developed by Roitberg and Mangel (1993). Their theory predicts that, for a given clutch size, the optimal probability of larval movement from the mother's perspective is always greater than or equal to the optimal probability of movement from the offspring's perspective. If travel is perceived to be too costly by the mother, then this means that she may produce a clutch size that does not appear optimal in terms of maximizing her lifetime fitness (i.e., a clutch too large to be supported by the resource). Behavioral responses by females that appear less than optimal because the cost of oviposition choice is too high (i.e. travel in fragmented landscapes) may be offset by mobile larvae that can disperse to nearby patches. Of course, the consequence of this strategy depends on how landscape fragmentation impacts successful larval dispersal. There is some evidence to suggest that, even when resource amount seems sufficient, there may be detrimental effects of fragmentation. In the resource amount/fragmentation study there were less adults at 35 days post-oviposition in the 6 egg treatment with two additional (more resource, fragmented) patches than in the 6 egg treatment with only one additional patch (less resource). Obviously, delay in reaching the adult stage results in negative fitness consequences for females as well as detrimental population consequences.

This study is one of the first to examine concurrently the influence of landscape structure on two ecological functions, movement and selection of reproductive sites. I provide evidence that red flour beetles perceive the patchiness of landscapes differently depending on the activity in which they are engaged and that a temporal shift in behavior may occur that changes sensitivity to the spatial scale of patchiness. Results of this study suggest that females modify oviposition site selection in response to complex factors which may include amount and spatial perception of resource, travel costs, and edge effects. I suggest that study of preference-performance in red flour beetles in relation to landscape structure should prove a fruitful area of research as current knowledge is very limited. More importantly, results of this study demonstrate how landscape structure may impact ecological functions differently, such as movement and oviposition site choice, and that these impacts may depend on behavioral responses that change with the temporal and spatial scales under examination.

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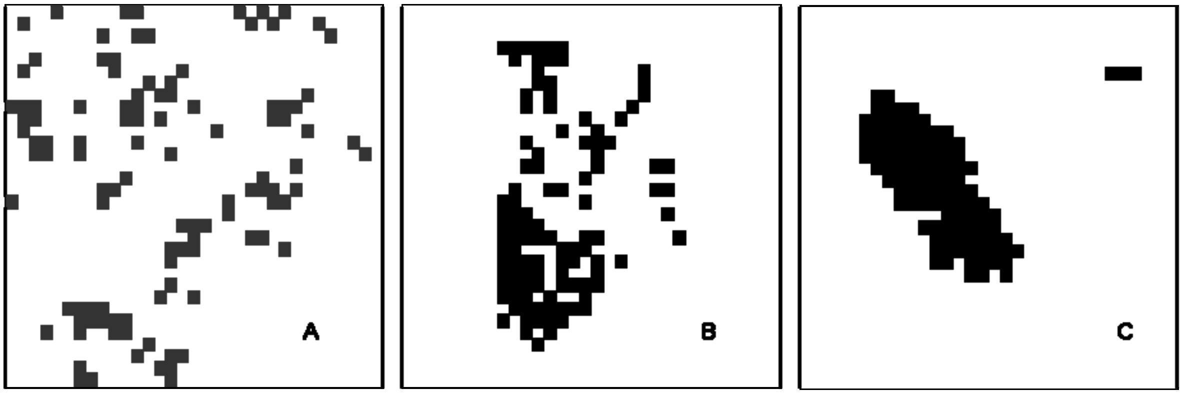

Figure 4-1 Three fractal landscape treatments, each with 108 habitat cells, but different levels of habitat fragmentation: A) highly fragmented (H = 0.9), B) intermediately fragmented (H = 0.5), and C) clumped (H = 0.2). Habitat cell grain size = 2 x 2 cm and landscape extent = 64 x 64 cm.)

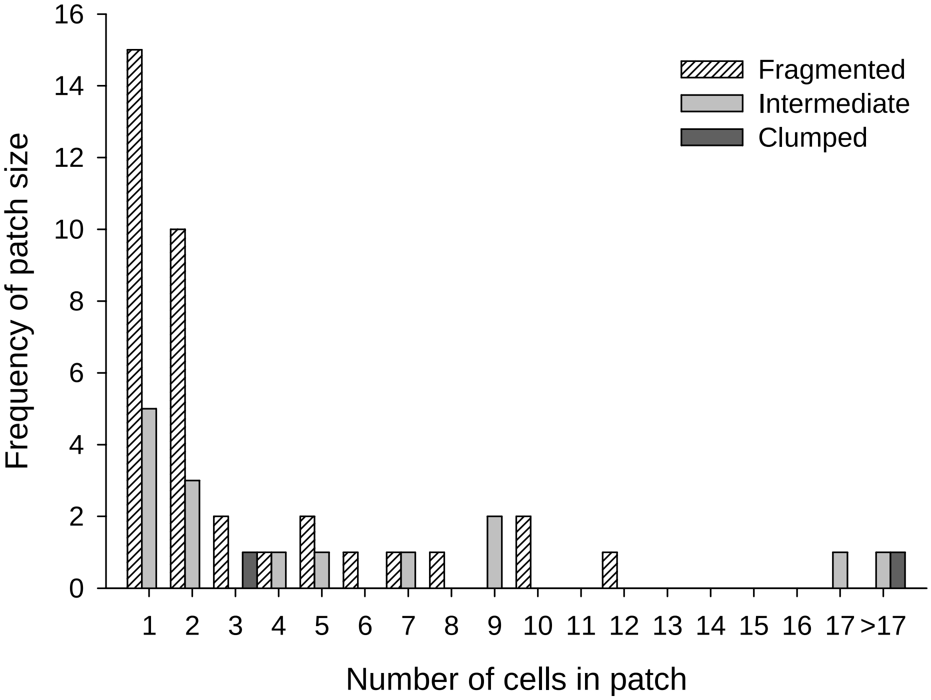

Figure 4-2 Frequency distribution of patch sizes on experimental landscapes with different levels of fragmentation.

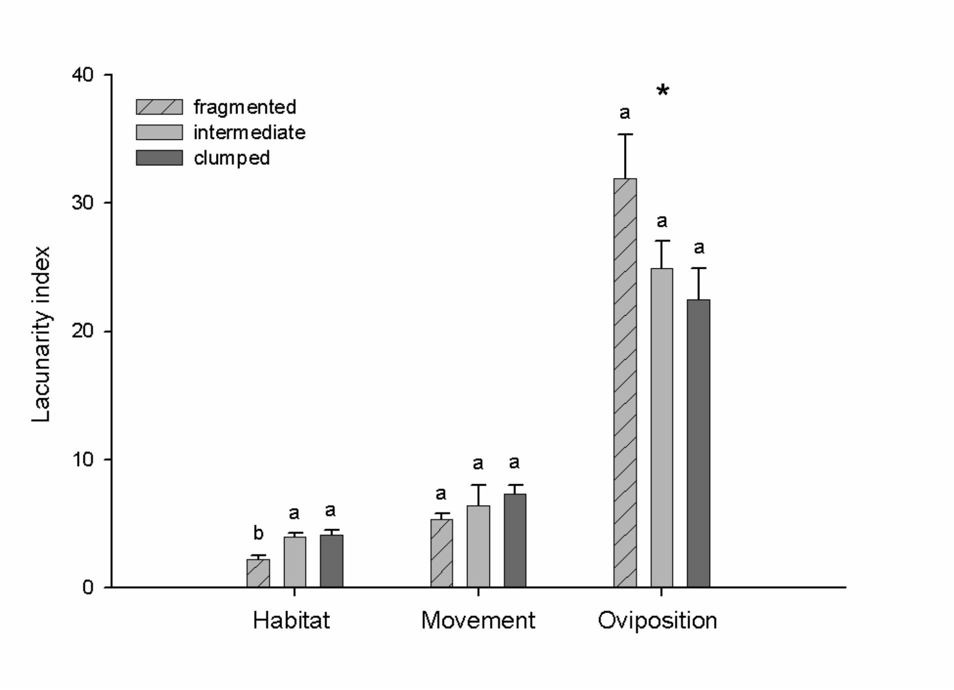

Figure 4-3 Mean + SEM of lacunarity index values for distributions of habitat cells, red flour beetle (*T. castaneum*) movement, and oviposition sites (cells with eggs). Bars with same upper case letters have means that are not significantly different among types of lacunarity or among landscape fragmentation levels. Bars with same lower case letters within an x-axis category have means that are not significantly different among landscape fragmentation levels (ANOVA, GLM procedure, alpha = 0.05).

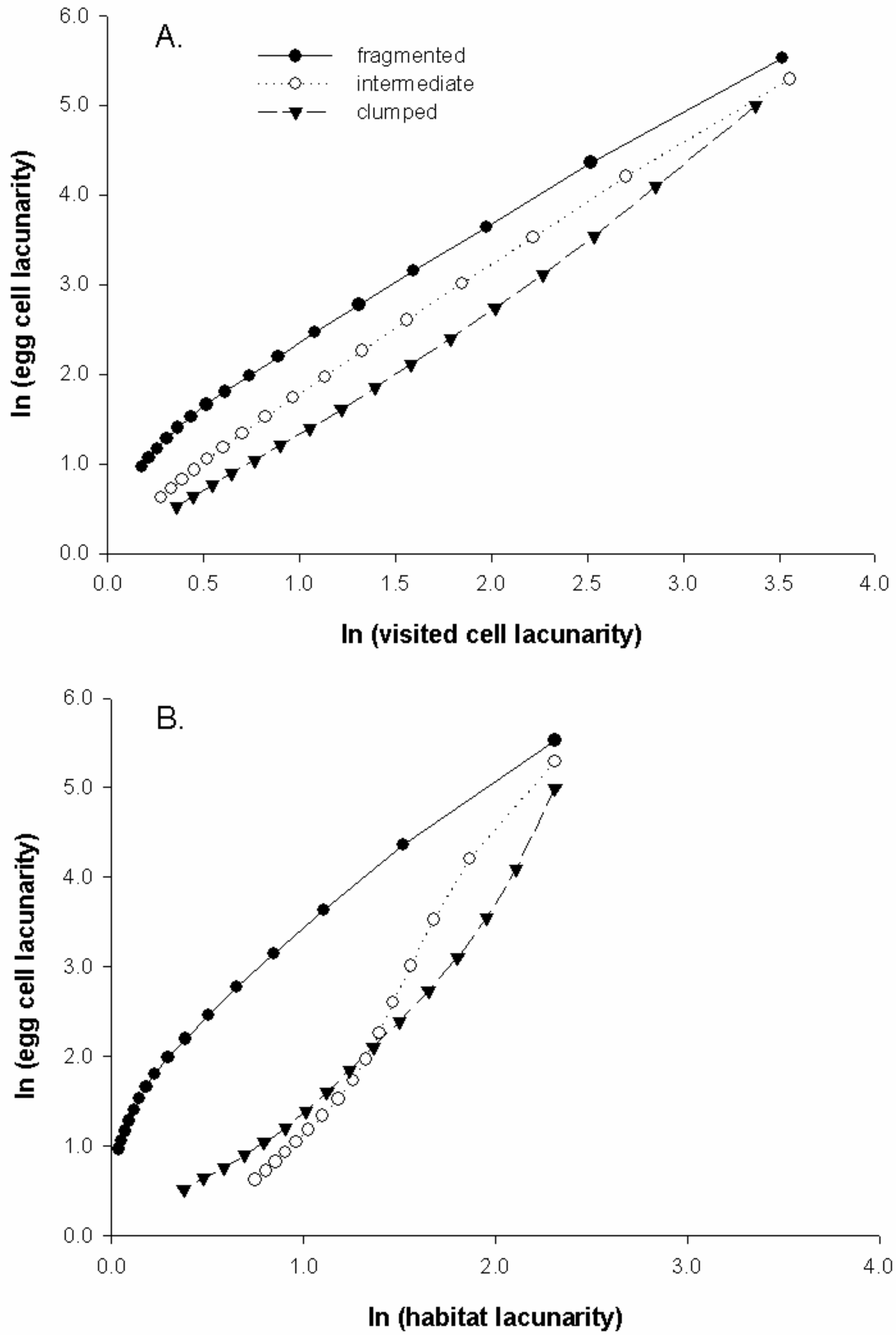

Figure 4-4 Regression plots of (A.) cells with one or more eggs versus visited cell lacunarity, and (B.) cells with one or more eggs versus habitat lacunarity.

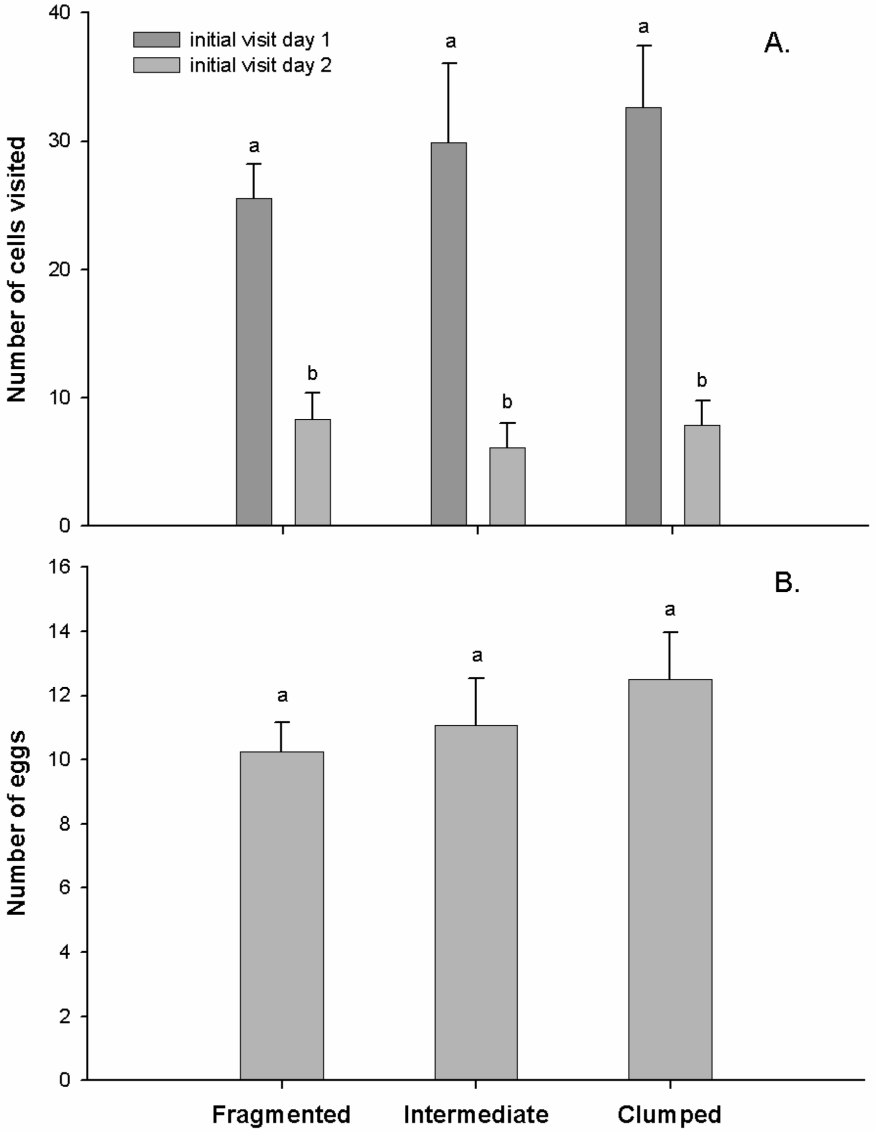

Figure 4-5 Mean + SEM of (A.) habitat cells (2 x 2 cm) first visited on first or second day, and (B.) eggs laid by red flour beetles (*T. castaneum*) over 48 hours on landscapes with different levels of fragmentation. Within a graph and bar color, mean bars with same letters are not significantly different (ANOVA, mixed procedure, alpha = 0.05).

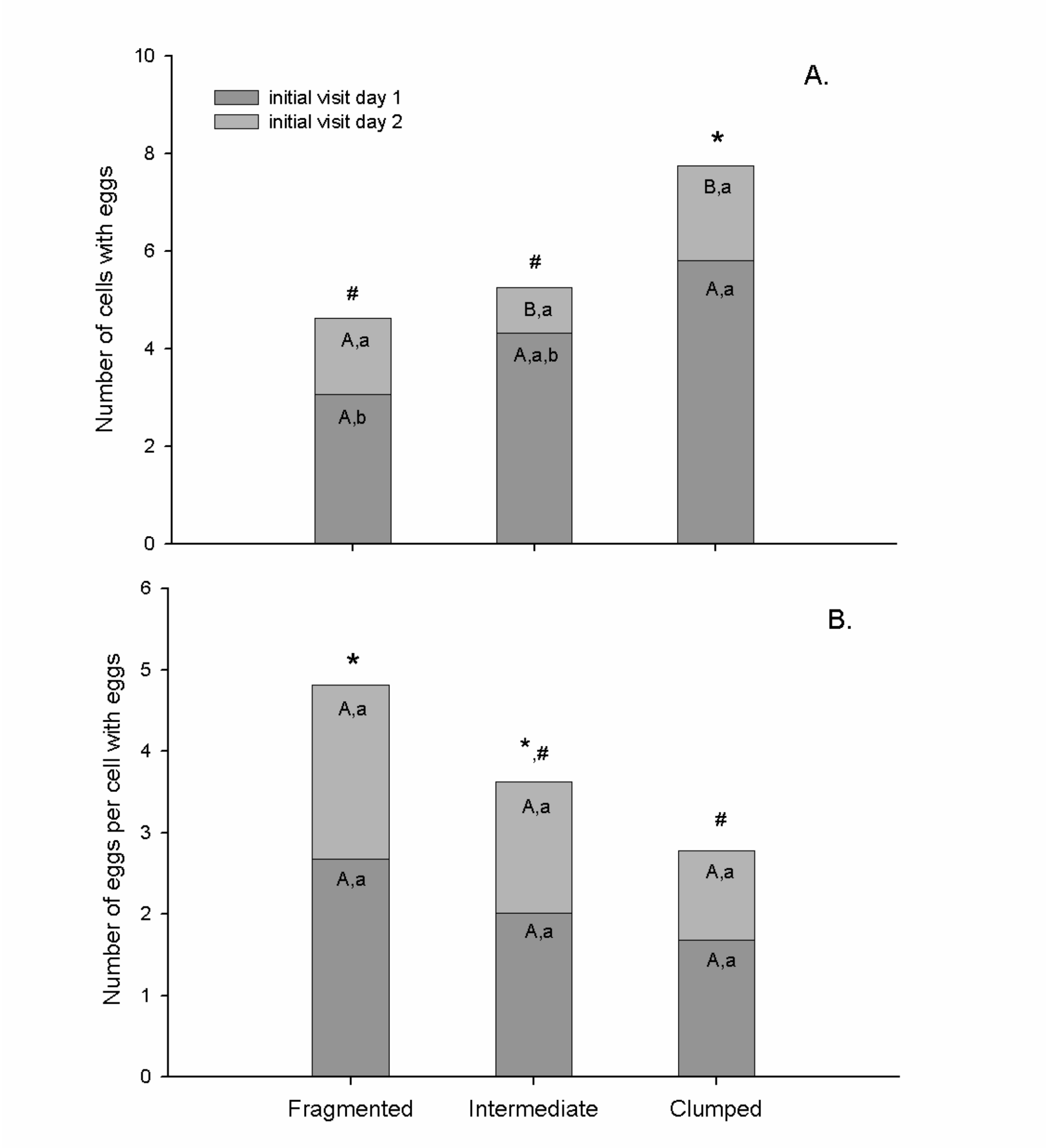

Figure 4-6 Mean number of (A.) habitat cells with one or more eggs and (B.) eggs per habitat cell with eggs in relation to fragmentation level of the landscape for cells initially visited by red flour beetles (*T. castaneum*) on day 1 or day 2. Bars with same upper case letter have means that are not significantly different between days within a fragmentation level. Bars with same lower case letter have means that are not significantly different among fragmentation treatments within a day. Stacked bars with the same symbol have combined means for both days that are not significantly different among landscape fragmentation treatments (ANOVA, mixed procedure, alpha = 0.05).

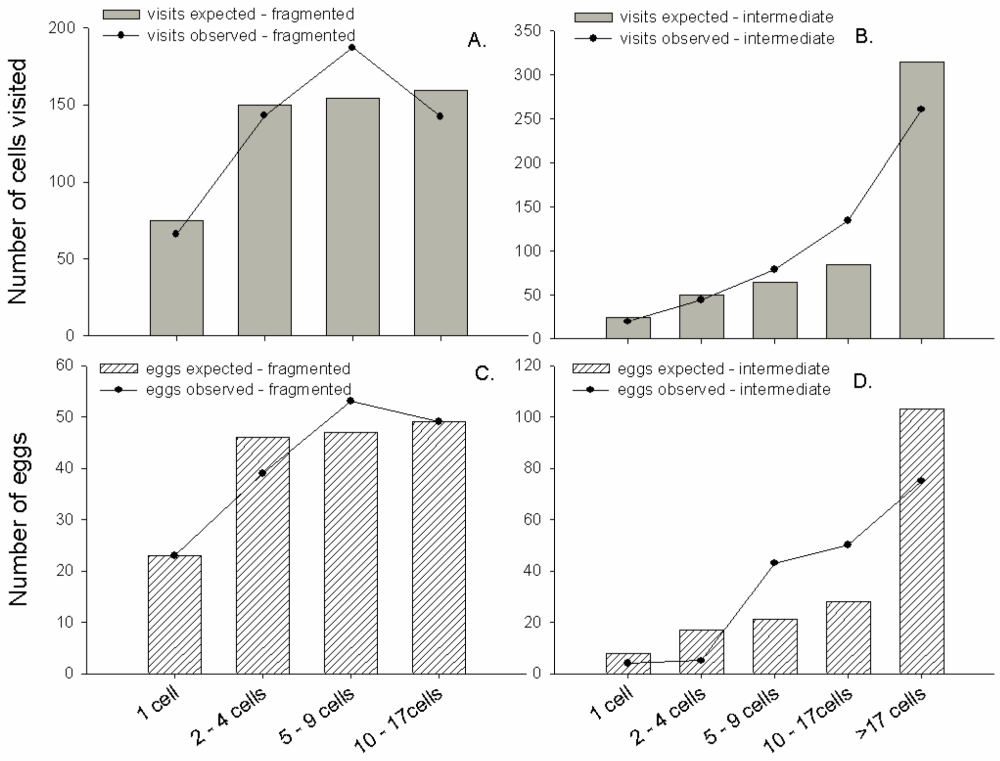

Figure 4-7 Total number observed versus expected for cells visited by red flour beetles (*T. castaneum*) in relation to patch size in fragmented (A.) and intermediate landscapes (B.) Total number observed versus expected for eggs laid in relation to patch size in fragmented (C.) and intermediate (D.) landscapes. Number of visits and eggs observed are not significantly different from expected in fragmented landscapes, but are significantly different in intermediate landscapes (Chi-square, Pearson's exact test, alpha = 0.05).

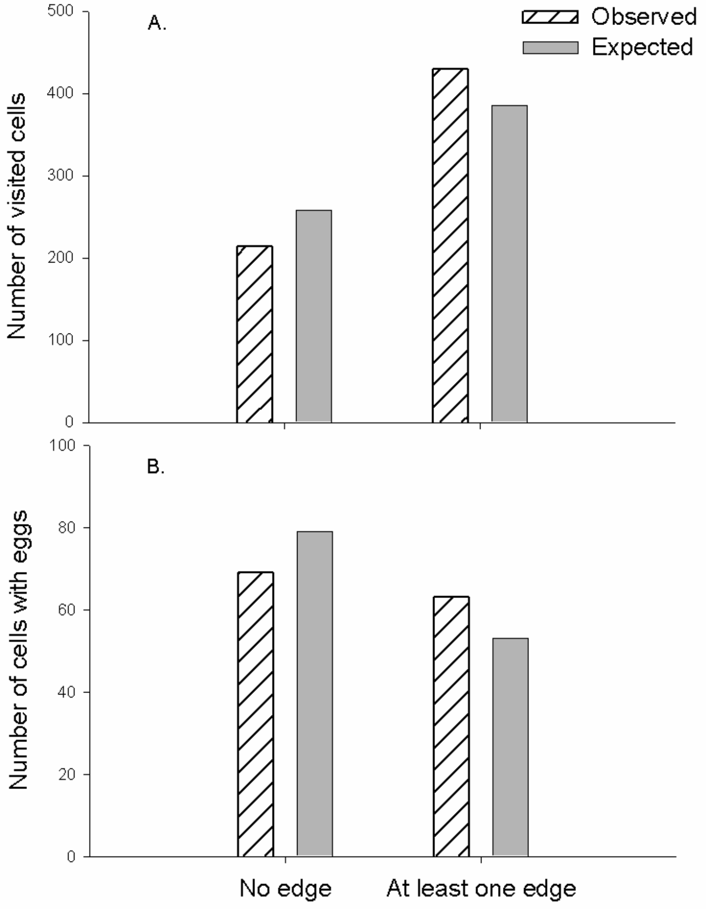

Figure 4-8 Number of observed vs. expected cells having at least one edge or no edge for A.) visited cells, and B.) cells with eggs in the clumped landscape. An edge is defined as a matrix cell adjacent either laterally or diagonally to the cell of interest. Cells expected were based on proportions of cell type in the landscape (40% - no edge and 60% - at least one edge). There was a significant difference in the number of observed vs. expected for no edge or at least one edge for visited cells but no significant difference for cells with eggs (Chi-square analysis of association, Pearson's exact test, alpha = 0.05).

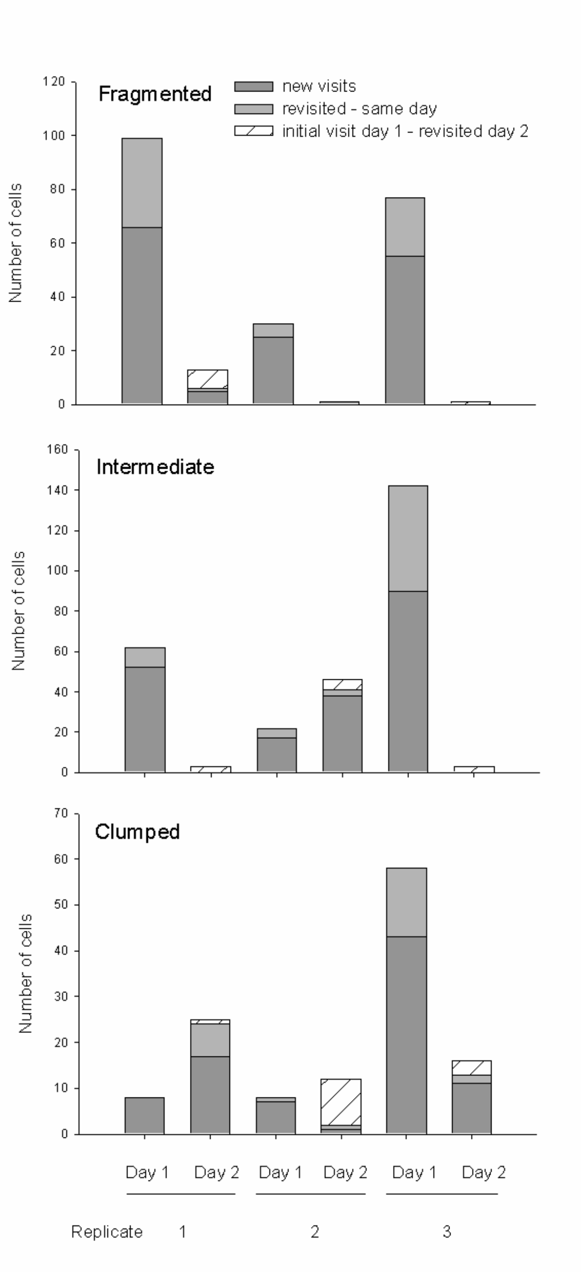

Figure 4-9 Results of video tracking red flour beetle(*T. castaneum*) movement showing total number of cells visited by beetles in first and second 24 h periods in fragmented, intermediate, and clumped, landscapes.

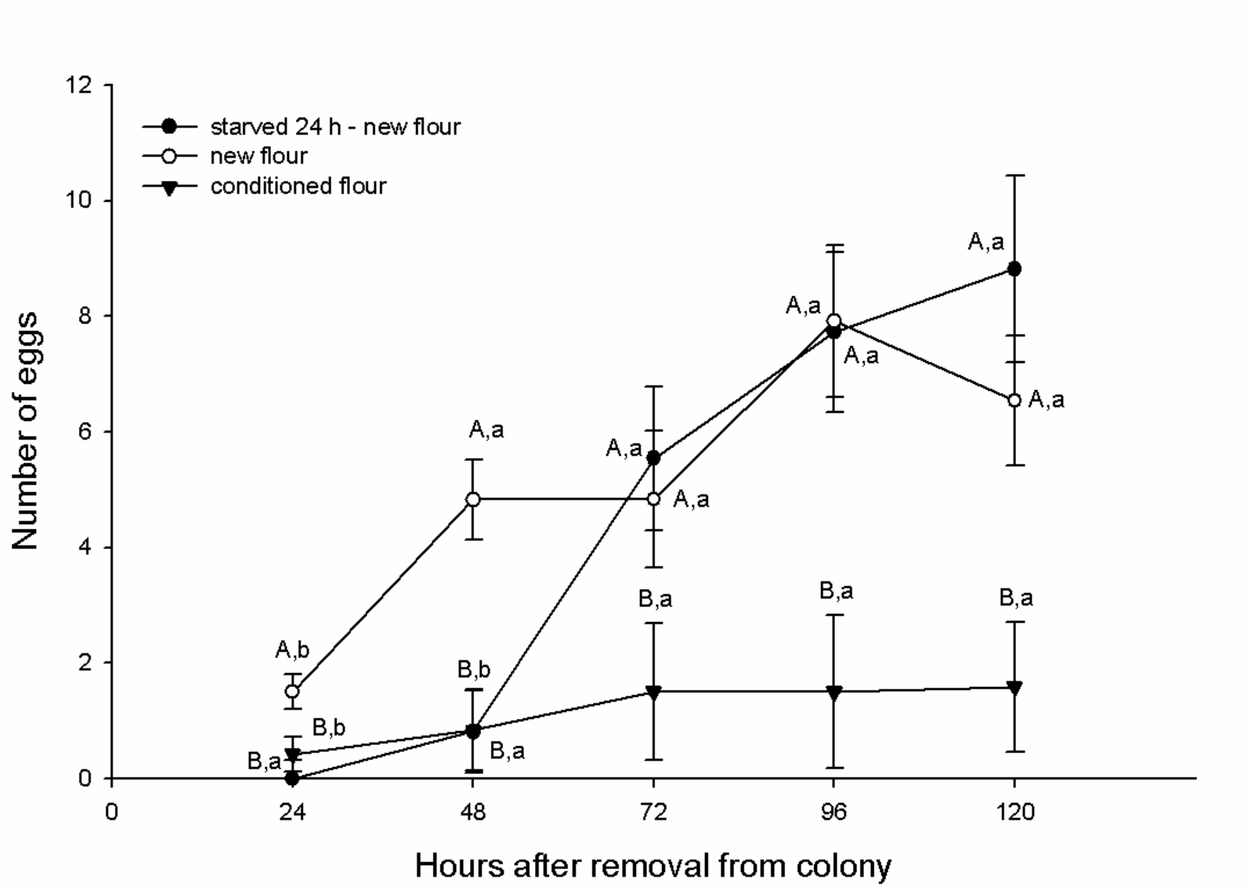

Figure 4-10 Mean ± SEM eggs laid per day by red flour beetle (*T. castaneum*) as a function of flour treatment and hour after removal from colony. Flour treatments include: placing beetle into new flour after a 24 h period with no flour, placing beetle into new flour, and placing beetle back into conditioned flour from the original colony. Symbols with the same upper case letters represent means that are not significantly different among treatments within an hour category. Symbols with the same lower case letters represent means that are not significantly different among hours after removal from colony within a flour treatment (ANOVA repeated measures, mixed procedure, alpha = 0.05).

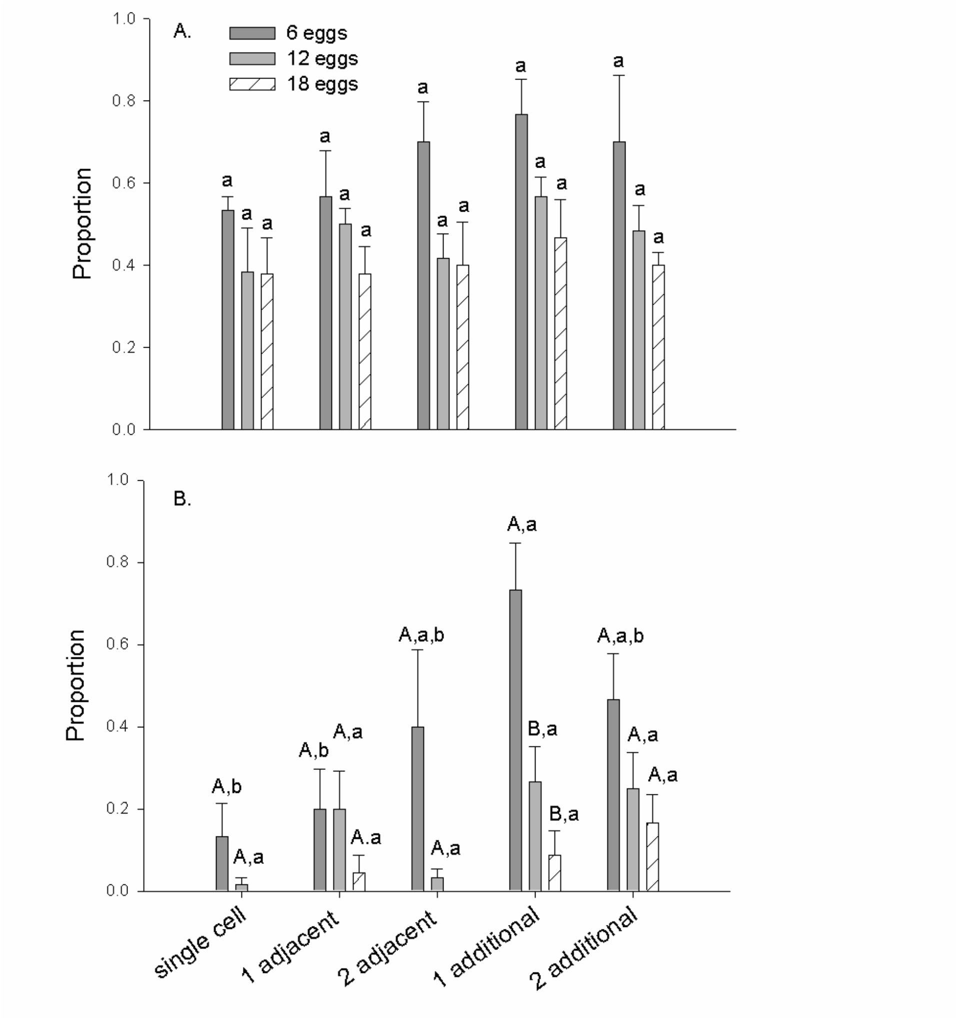

Figure 4-11 Proportion of all red flour beetle (*T. castaneum*) life stages (adult, pupa, and larvae) (A.) and adults only (B.), surviving to 35 days post oviposition. Patch treatments consist of a single cell with eggs (each cell = 2 cm^2 = 0.04 g), a single cell with eggs plus one adjacent cell, and a single cell with eggs plus two adjacent cells, a single cells with eggs plus one additional cell placed 15 cm distant, and a single cell with eggs plus two additional cells all 15 cm distant from one another. Bars with same upper case letters within a patch treatment have means that are not significantly different. Bars with same lower case letters have means that are not significantly different among egg density treatments (ANOVA, mixed procedure, alpha = 0.05).

Table 4-1 Results of fitting regression curves, produced by plotting *ln* habitat cells, and *ln* visited cells versus *ln* egg cell lacunarity, to best – fit linear equation for three landscape patterns.

Visited cell vs. oviposition site lacunarity

Landscape	*Best – fit equation*	r^2	*F - value*	*95% CI*
Fragmented	power (a.b.c)	0.999	9666.304	1.460 – 1.657
Intermediate	power (a,b,c)	0.999	164410.916	1.707 – 1.772
Clumped	exponential (a,b,c)	0.999	96658.020	5.957 – 7.1287

Habitat cell vs. oviposition site lacunarity

Landscape	*Best – fit equation*	r^2	*F - value*	*95% CI*
Fragmented	$y^{0.5} = a + bx^{0.5}$	0.999	14852.362	0.923 – 0.989
Intermediate	$y^{-1} = a + b\ln x/x$	0.997	5133.346	-1.957 - -1.788
Clumped	exponential (a,b,c)	0.999	9596.276	-1.491 - -1.262

Table 4-2 Mean + SEM of length (cm) of elytra of red flour beetles (*T. castaneum*) surviving to adulthood in different amounts and spatial arrangement of flour resource and egg densities. Resource treatments include: a single cell (2 cm^2) with eggs, a single cell with eggs and one adjacent cell, a single cell with eggs and 2 adjacent cells, a single cell with eggs with 1 additional cell 15 cm distant, and a single cell with eggs and 2 additional cells, all separated by 15 cm.

Resource treatment	n	No. eggs	n	No. eggs	n	No. eggs
		6		12		18
Single cell	4	2.03 + 0.05	1	1.94 + 0.00	0	0.00 + 0.00
1 adjacent	6	2.04 + 0.11	12	1.97 + 0.08	4	2.03 + 0.04
2 adjacent	12	2.02 + 0.04	1	1.89 + 0.00	0	0.00 + 0.00
1 additional	20	2.05 + 0.04	11	1.98 + 0.04	9	2.01 + 0.04
2 additional	14	2.00 + 0.04	14	2.01 + 0.04	7	1.98 + 0.05

Conclusions

Knowledge of movement behavior of insects in patchy landscapes is essential for understanding their distribution and movement patterns, modeling dispersal and population structure, and developing biologically- and biodiversity-based integrated pest management programs. Research on movement behavior using experimental landscape models has been conducted over the last several decades to further develop theory as well as provide information on how specific species respond to landscape structure. Results of my dissertation research add to this growing body of knowledge, providing not only knowledge that will aid developing theory on the subject of insect dispersal, but also information on red flour beetle movement behavior that may eventually lead to improved management practices for this pest.

My initial experiment (Chapter 2) showed that red flour beetle responds to differences in landscape structure resulting in modification of search behavior. Lacunarity analysis revealed that distributions of movement pathways on random landscapes differed significantly from landscape pattern with movement more aggregated than the underlying habitat, especially in coarse-grained landscapes. There was a dramatic and abrupt increase in lacunarity values (more aggregated) between the coarse-grained landscape and the fine and intermediate-grained landscapes. Movement pathway metrics supported the finding of the lacunarity analysis, showing that beetles accessed similar proportions of all landscapes, but were searching fine-grained landscapes quite differently than coarse-grained landscapes. In fine-grained landscapes, beetles moved more slowly and tortuously and this behavior resulted in less displacement and increased retention time. The fine-grained landscapes were more fragmented containing many small patches. Thus, they had many more patch edges than the coarse-grained landscapes. Fine-scale responses to edges in fine-grained landscapes may be responsible for increased pathway complexity as well as increased retention time because beetles spent more time in individual habitat cells on fine-grained landscapes. Values for distribution of movement pathways and parameters in intermediate-grained landscapes were intermediate between the fine and coarse-grained landscapes. Movement pathways had a more aggregated distribution on coarse-grained landscapes indicating that beetles moved in a linear fashion. Pathway metrics showed that movement on coarse-grained landscapes was faster and straighter; therefore, displacement was higher than on the fine-grained landscapes. Beetles appeared unable to perceive the aggregated habitat on coarse-grained landscapes that had large interpatch distances compared to the fine-grained landscapes. The low viscosity of this landscape resulted in low retention time in these landscapes.

Because observations in Chapter 2 showed that beetles modified search behavior depending on landscapes structure, in Chapter 3 I addressed questions concerning how red flour beetle gathers information on the distribution and quality of habitat while moving on landscapes. Results of the experiment designed to determine perceptual range indicate that beetles do not perceive habitat unless it is in close physical proximity, suggesting that beetles moving on landscapes may have little information concerning the distribution of habitat at distances larger than a few centimeters. This result coincides with observations in Chapter 2 indicating that beetles did not perceive habitat in coarse-grained landscapes and provides further support to the premise that red flour beetle do not detect patches until a systematic search strategy brings them in physical proximity to the patch edge. Hunger level and air flow did not increase the number of beetles able to find a resource patch from various distances. It is possible that beetles could have perceived the patch but were not motivated to contact the resources or were constrained by some other behavior at the time of the experiment.

The second part of Chapter 3 examined the response of beetles to patches with different boundary characteristics. Beetles clearly perceived differences in the boundaries of patches as indicated by movement pathways and measures of permeability. Results presented in this chapter also support my contention that beetles do not evaluate patches until direct contact has been made because significant differences in movement pathway response to boundary treatments only occurred in the area immediately surrounding the patch. Beetles entered sheltered patches (low resource) more quickly and directly than high-edge (more resource) and low-edge (low resource) patches. Permeability of high-edge patches changed dramatically with additional encounters, suggesting that full evaluation of patches probably occurs after beetles enter the patch. At initial contact, the high-edge patches were much less permeable than the low-edge patches, but this changed over time so that the two became more equal. Once beetles were inside the patch, the difference in probabilities of exiting the patch became larger between the high and low-edge patches, 50 vs. 20% respectively. Significant differences in retention time between high-edge and low-edge patches further illustrate how edge effects and/or resource amount may impact colonization rates and movement among patches. Red flour beetles appear to respond thigmotactically to both patch and structural edges and this tendency could result in increased aggregation in areas of high edge concentration.

Chapter 4 examined two important ecological functions concurrently - movement and oviposition - in response to landscape structure. This study demonstrates that beetles movement response to landscape structure may depend on the activity in which they are engaged. Distribution of movement pathways was similar to that of the underlying habitat pattern; but in contrast to movement, distribution of oviposition sites was more aggregated than the pattern of habitat. For red flour beetles, selection of oviposition sites appears to be modified by the underlying habitat pattern as well as by the pattern of previously visited locations. The functions of movement and oviposition were shown to have discontinuity in time that perhaps influenced beetles perception and use of the landscape. Beetles visited more habitat on the first day of the experiment than on the second day and likely did not begin ovipositing until ~ 24 h after the start of the experiment. Results indicated that beetles returned to lay eggs in locations previously visited on the first day on clumped and intermediately-fragmented landscapes, but did not follow this behavior in highly fragmented landscapes. Beetles laid fewer eggs in more locations on the clumped and intermediately-fragmented landscapes and placed more eggs in fewer locations in highly fragmented landscapes. These observations suggest that beetles traveled more on clumped and intermediately-fragmented landscapes and less on fragmented landscapes. In general beetles preferred to oviposit in larger patches having more than adequate resources for survival of progeny. Overall results of this study suggest that females modify oviposition site selection in response to complex factors which may include amount and spatial perception of resource, travel costs, and edge effects.

This work provides important information on how red flour beetle perceives and responds to landscape structure at relatively fine scales. Previous research on the study of movement behavior in experimental landscapes have cautioned against applying quantitative extrapolation of information gained at fine scales to broader scales because movement behavior may change dramatically with an increase in both spatial and temporal scale. This work corroborates earlier studies suggesting that changes in movement patterns over space and time make predictions concerning population distribution difficult. However, inability to make quantitative predictions does not prevent information on movement behavior gathered at relatively fine scales to be utilized for comparative and qualitative assessments of how movement of a species may be influenced by particular habitat patterns. Data gleaned from studies of this nature may enable future management efforts to be fine-tuned the according to the structure of a particular landscape. For instance, several inferences may be made about where to concentrate control efforts for red flour beetle as a result of this research. For example, this work indicates that red flour beetle is attracted to and prefers to oviposit in larger amounts of resource and shelter may enhance this preference. The probability of emigration from such a resource is less than from smaller patches in open areas. Beetles may be constrained or aggregate in areas where they can move easily among many patches. Several behavioral characteristic that influence dispersal ability were identified in the current studies. Beetles appear to have a thigmotactic response to vertical edges that could influence areas of movement or colonization. Despite appearing to having a rather myopic perceptual range, red flour beetles seem to have good gap-crossing abilities, although more research is needed on dispersal range, which remains unknown. Results of this work suggest that future studies on movement of red flour beetles should examine how behavior may change over larger spatial and temporal scales as well as how various endogenous states, such as sex, age, and reproductive status influence movement behavior.

In summary, this research has contributed to a better understanding of the behavior of red flour beetle during two critical ecological processes: movement and oviposition. Insights were gained concerning how red flour beetle perceives resources, modifies search strategies, responds to boundaries, and chooses reproductive sites in patchy landscapes. This work provides fundamental information that contributes to understanding movement patterns of red flour beetles which may be useful for modeling their dispersal and improving integrated pest management programs for their control.