Population dynamics and resource utilization of the lesser grain borer, *Rhyzopertha dominica* (F.)

by

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Abstract

Genomic tools can provide important insights into the biology, dispersal, and feeding habits of insects whose behavior is difficult to monitor in the field, including stored product insects that primarily attack dried stored commodities and disperse throughout different agricultural landscapes where they may feed on alternate food resources. The lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae), is a cosmopolitan pest that primarily attacks stored grain, but can be found in prairie landscapes despite the lack of primary food hosts in these natural locations. The presence of *R. dominica* in natural landscapes surrounding grain storage structures suggests that there could be interactions between individuals that colonize these habitats. In addition, lab studies have shown that this insect can feed and develop on alternative food hosts found in prairie landscapes. This thesis investigates the local interactions between populations of *R. dominica* at grain storage facilities and a nearby tallgrass prairie over the course of three field seasons using population genomics and dietary habits of *R. dominica* from two different tallgrass prairies (Nine Mile Prairie, Lincoln, NE and Konza Prairie, Manhattan, KS) using molecular gut content analysis.

The population structure of *R. dominica* was examined by generating single nucleotide polymorphisms for individuals over three years to determine the interactions between insects caught in a large-scale grain elevator, a research flour mill, and a native tallgrass prairie. All three locations were located within 10 miles of one another. Heavy admixture was observed between the three locations across all three years surveyed, and location, field season, and the interaction of these two variables failed to explain the majority of the variation in genotypic diversity found in this species. In fact, more variation was observed among insects caught within a single location relative to insects collected from different locations. The most divergent populations were observed in the 2019 field season, likely due to the high amounts of rainfall

observed and the lower number of individuals caught during this field season. The population of insects caught in the flour mill was also the most dissimilar to the flour mill and the prairies, which can be attributed to the strict guidelines for pest prevention in these types of facilities. The heavy admixture observed between the three locations, coupled with the low genetic variation between the locations, suggests significant interactions between these populations and indicates that *R. dominica* may be able to migrate between sources of grain and natural landscapes.

To identify gut contents of *R. dominica* from Konza Prairie and Nine Mile Prairie, beetles were collected during their active flight season and plant-specific *rbcL* primers were used to detect and taxonomically classify plant DNA found inside the insect guts. Overall, we were able to identify 57 unique plant sequences between the two locations and identify 27 different plant species within their guts. While some individuals at both locations had recently fed on stored product commodities, products derived from these hosts were in low abundance. The most common and abundant taxon found in guts of insects caught in both locations was classified as *Thinospyrum ponticum*, which is known as tall wheatgrass. Several other plant species that are native to both the Konza and Nine Mile Prairies were identified, suggesting that *R. dominica* is able to exploit natural resources.

The results from both studies highlight that *R. dominica* can exploit natural resources found in prairies and those that insects caught innatural landscapes near grain storage facilities interact with one another. These results contribute an increase in the understanding of the population dynamics of *R. dominica* between grain storage and the natural environment at a local scale, as well as their resource utilization. Previous studies have shown that populations in grain storage facilities can rebound quickly after fumigation and that insects in the surrounding landscape may serve as source populations. Our study confirms that insects from grain storage facilities and flour mills interact with those found in adjacent habitats. Therefore, we can use

these results to better inform pest management and prevention of infestations by accounting for beetles that migrate from natural landscapes. Future work should assess gut content structure of *R. dominica* found in grain storage in comparison to those caught in landscapes, as well as assess genetic population structures across other location types and systems to gain further insights into their movement and migration.

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Chapter 1 - Introduction and Literature Review

Abstract

Global crop damage and contamination caused by stored product insect pests after crop harvests accounts for yield losses ranging from 9- 20%. The lesser grain borer (*R. dominica*), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a prominent pest insect of stored products found throughout the world. Although *R. dominica* is mainly found in grain storage and food facilities, it is also extensively trapped and captured in other landscapes, such as tallgrass prairies and woodlands. Previous studies have indicated that the movement of *R. dominica* between grain storage facilities is usually facilitated by the transportation of infested grains between food facilities. However, the presence of *R. dominica* in other landscapes suggests they may exploit alternate food resources. This chapter provides a brief overview on the economic significance of stored product insects and the commonly used approaches for managing these pests, focusing on its biology and life history, food resources, flight and movement, and population dynamics. At the end of the chapter, I describe the objectives and framework of this research focusing on the population genetics and molecular gut content of *R. dominica* populations collected from Manhattan, KS. A better understanding of the *R. dominica*'s ability to immigrate into storage facilities and utilize resources found in tallgrass prairies can help researchers develop new strategies for managing this important stored product pest.

Introduction to stored product insects

Stored product insect pests cause tremendous yield losses due to their direct (e.g., feeding) and indirect (e.g., contamination) damages after crops are harvested from the field worldwide. The lesser grain borer (*R. dominica*), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is a prominent pest of stored products. It feeds on whole, undamaged grains, including wheat, sorghum, corn, beans, and rice, as well as finished products. This chapter

provides a brief overview on the economic significance of stored product insects and the commonly used approaches for managing stored product insects. Because my research focuses on *R. dominica*, this chapter also provides more detailed review on *R. dominica*, including its biology and life history, food resources, flight and movement, and population dynamics. At the end of this chapter, I describe the objectives and framework of this research focusing on the population genetics and molecular gut content analysis of *R. dominica* populations in Manhattan, KS, in the major grain-producing region of the United States.

Damage caused by stored product insects

With the global population projected to reach 9.8 billion people by 2050, food production must increase by 70% to sustain the predicted population (FAO, 2018). However, climate change, along with the increasing frequency of extreme weather events, is rapidly changing the agricultural landscape, leading to unprecedented challenges in crop production (Ray et al., 2013). Improving the durability of post-harvest agricultural commodities is of paramount importance to keep pace with the growing demand for food.

Insect feeding is one of the most common damages to post-harvest commodities, with insect damage accounting for around 10% of damage to dried, stored commodities worldwide and losses reaching up to 20% in developing countries (Phillips and Throne 2010). Products that stored product pests can exploit range from raw grain to high-value finished commodities, including flour, dried pet foods, and other grain-based products. Damage to these products occurs predominantly from insect feeding, but frass, exuviae, and other byproducts of insect activity can render infested products unsuitable for human and animal consumption. In addition, insect feeding activity can generate moisture, promoting fungal and microbial growth. Insects can also spread fungal spores and microbes as they move through grain storage (Atanda S. A, 2011).

Common pest species of stored products belong to a range of diverse taxonomic lineages, including beetles (order Coleoptera), moths (order Lepidoptera), and mites (subclass Acarina) and are commonly categorized as either primary or secondary pests. Primary pests feed on whole, undamaged grain, while secondary pests feed on damaged and milled commodities (Rees, 2004). Damage created by primary feeders can create niches suitable for colonization by secondary feeders, leading to compounding insect damage (Shah et al., 2021).

Stored grains have been vulnerable to insect infestations since the inception of agriculture and storage of commodities over 10,000 years ago (Rees, 2004). Currently, stored products are vulnerable to insect infestation at every step within the post-harvest supply chain from the field to the markets where they are sold to consumers. Once insects infest commodities and enter the supply chain, they persist, and their population levels quickly expand because they are sheltered from environmental conditions and have access to abundant food resources. For example, grain is typically stored in large reserves, including silos that can hold up to 800 tons of product and large elevators that can hold up to 50,000 bushels of grain (Phillips and Throne, 2010). Moreover, stored product insect populations from landscapes immediately adjacent to food facilities and storage structures easily infiltrate these structures. Additionally, it is difficult to trace the origins of infestations and their spread due to frequent global trade of raw and finished commodities and because many species of stored product pests have been established globally for decades (Plarre and Burkholder, 2009).

Management of stored product insects

The ability to control and manage stored product pests is imperative because their populations can increase 10-fold per generation under suitable conditions (Hagstrum and Subramanyam, 2006). There are many methods of controlling stored product infestations, including biological control, insecticides, fumigation, and sanitation (Hagstrum and

Subramanyam, 2006). Fumigation is one of the most common methods to treat stored product infestations in large storage and processing facilities. The broad-spectrum fumigant methyl bromide was once used extensively to treat stored product insect infestations; however, its usage has been phased out due to its ozone-depleting nature (UNEP, 1990). Phosphine has largely replaced methyl bromide for treating infestations (Collins, 2006; Opit et al., 2012). Its usage is prevalent due to its low cost, ease of application, and efficiency at targeting stored product pests (Nayak et al. 2020). Although phosphine can still be successfully deployed to manage stored product insect infestations, many species and populations of stored product insects have developed resistance to this fumigant after decades of use (Chaudhry, 2000). Though these methods of integrated pest management (IPM)can be effective, they are reactive treatments of established pest populations, as opposed to proactive treatments that prevent infestation of storage structures and food processing facilities.

Additionally, these reactive measures are generally implemented after significant economic damage has occurred and do little to prevent insect re-infestation of stored products after fumigation. In fact, insect populations often rebound after fumigation, presumably by insects living in small spillage piles outside these facilities or insects from the surrounding landscape (Campbell and Arbogast, 2004).

Biology and life history of lesser grain borer

One stored product insect that has developed resistance to phosphine is the lesser grain borer (*R. dominica*), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). A report by the Food and Agriculture Organization of the United Nations on global pesticide susceptibility of stored product insects indicated that of 92 field populations of *R. dominica* surveyed, 22 were resistant to phosphine (V F Wright et al., 1990). Other subsequent studies have identified resistant populations in the United States, Australia, and Pakistan (Afful et al., 2018; Collins, 2006;

Nayak et al., 2020; Wakil et al., 2021). *Rhyzopertha dominica* is a cosmopolitan pest found worldwide in temperate and tropical areas (Perez-Mendoza et al., 1999). It is a primary pest of various stored commodities, with wheat, rice, sorghum, and corn serving as their predominant hosts. Although the species was thought to have evolved in India, *R. dominica* has had devastating impacts on agriculture throughout the world. For example, *R. dominica* became prevalent in the United States during World War I from wheat imports from Australia (Schwardt, 1933).

Under laboratory conditions, *R. dominica* can complete its life cycle in 25 days at 34°C and 14% grain moisture (Edde, 2012; Schwardt, 1933). Adults can live up to 120 days and have been observed to live up to a year in some laboratory studies (Edde, 2012). Once larvae hatch, they bore into grain kernels and feed until they pupate. After pupation, adults emerge and are 2-3 mm in length and reddish-brown in color. Adults are cylindrical in shape and possess a distinct serrated pronotum that covers their heads. Their elytra are distinguishable by their coarse puncture pattern on their exoskeleton (Edde, 2012; Koehler and Pereira, 2012).

Food resources used by *R. dominica*

Rhyzopertha dominica belongs to the family Bostrichidae, which contains many wood-boring powder post beetles and two major pests of stored products, including *R. dominica* and larger grain borer, *Prostephanus truncatus* (Horn 1878). Though *R. dominica* is primarily a pest of whole grain and is found in facilities where raw and processed commodities are stored, this insect is often caught in natural landscapes, including prairie and forest habitats (Edde, 2012; Jia et al., 2008; Mahroof et al., 2010). These habitats may serve as resource patches for *R. dominica* as they traverse the landscape in search of larger caches of suitable food resources.

Alternatively, like other members of the family Bostrichidae, they may be able to exclusively subsist on food resources in these habitat patches, allowing them to persist in these

landscapes and serve as potential source populations for infestation of nearby storage and food processing facilities. Laboratory studies have shown that *R. dominica* could survive and reproduce on acorns (Edde and Phillips, 2006; Jia et al., 2008; V F Wright et al., 1990). Additionally, although short-term survival was documented on fresh-cut twigs, dried plum fruits, and peanut seeds, no *R. dominica* progeny emerged from any of these food sources after 90 days of observation (Wright et al. 1990). In concert, Edde and Phillips (2006) documented the attraction of *R. dominica* to volatiles from several non-host plants, including acorns and potatoes, in conjunction with the *R. dominica* male aggregation pheromone. Despite this evidence from laboratory studies, it is currently unknown if *R. dominica* feeds on these resources in the field. However, an understanding of alternate host utilization can lead to the development of preventative management and monitoring programs for *R. dominica* that may be entering grain storage from the natural landscapes (Daglish et al., 2017; V F Wright et al., 1990).

Flight and movement of *R. dominica*

Many prior studies have documented the movement of *R. dominica* through several different types of natural landscapes. *Rhyzopertha dominica* adults are known to be strong fliers capable of covering long distances in short periods (Daglish et al., 2017; Dowdy, 1994; Edde et al., 2006; Holloway et al., 2020). In a study by Mahroof et al. (2010), *R. dominica* adults were released and recaptured in wooded areas and open sites. In wooded areas, the mean dispersal distance of recaptured insects ranged from 337 m to 375 m, and in open sites, the mean dispersal distance of recaptured insects ranged from 261 m to 333 m away from the release sites (Mahroof et al., 2010). Furthermore, Ridley et al. (2016) documented dispersal from grain facilities of up to 100 m, while other studies have documented flight behavior outside grain storage facilities (Dowdy and McGaughey, 1994; Leos-Martinez et al., 1986; Toews et al., 2006). Collectively,

these studies show that *R. dominica* can disperse through different landscapes to other sources of grain.

The initiation of flight is influenced by physiological status and life history aspects of *R. dominica*. Younger beetles had a greater tendency to initiate flight and made up a majority of migrating beetles (Aslam et al., 1994; Edde, 2012). Of *R. dominica* caught leaving grain silos, many were fecund mated females, which suggests that, in some cases, females will seek out new suitable food sources to lay their eggs. A single adult *R. dominica* can produce an average of 242 offspring (Daglish et al., 2017; Ridley et al., 2016), so these emigrating females can potentially serve as sources for new infestations. In laboratory studies, female beetles were more frequently caught in traps baited with the male aggregation pheromone, suggesting that potentially mated female beetles are more prone to migrate in search of an attractive cue (Cogburn et al., 1984; Dowdy, 1994). These factors indicate that young, mated females are more prone to flight and migration and perpetuate infestations.

Habitat and environmental factors also impact flight initiation. Low food quality and high population densities have been associated with significant increases in flight initiation of *R. dominica* (Perez-Mendoza et al., 1999, 1998). Since *R. dominica* larvae primarily feed internally and pupate within intact grain kernels, high-density populations with low food quality are not ideal for development. Food deprivation for one day also caused flight initiation in *R. dominica* (Mahroof et al., 2010; Perez-Mendoza et al., 1999). Environmental factors can also influence flight propensity, with temperature having a significant impact on flight activity (Ching'oma et al., 2006; Dowdy, 1994; Edde et al., 2006). *Rhyzopertha dominica* adults are more active in warmer months; however, in the winter season, they are not typically detected or caught in Lindgren flight traps in geographic areas where temperatures drop below freezing (Dowdy and McGaughey, 1994; Edde et al., 2006). These habitat and environmental factors could drive *R.*

dominica through the natural landscape in search of a different food resource, with warm temperatures allowing for maximum fight capability.

Population dynamics of *R. dominica*

A previous study by Cordeiro et al. (2019) documented the source-sink dynamics of *R. dominica* and the impact these dynamics had on population structure across the United States. Two major genotypes were found throughout various states of the United States, but up to seven genotypes were identified in the Great Plains, where grain is stored in higher volumes compared to other regions in the United States. The main contributor to the population structure across this broad geographic area was human-aided movement and transportation of grains, with populations of insects from the Great Plains serving as sources for populations in other parts of the United States. Although this study examined broad population structure across a large geographic region, smaller and more local interactions among insect populations in neighboring facilities and adjacent landscapes were not assessed.

The gene flow between populations of *R. dominica* collected from different geographical locations across Turkey was shown to be comparatively less than that observed in the United States and Australia (McCulloch et al., 2020). The authors hypothesized that the reduced gene flow is due to more localized agricultural practices in Turkey, where grain is stored and processed closer to where it was harvested, and to the mountainous landscape of Turkey, which may impede movement. One additional difference between the McCulloch et al. (2020) and Cordiero et al. (2019) studies is that the former study was performed over a smaller geographic scale, which likely allowed the authors to observe population differences at a finer scale.

Objectives and framework of this research

Though *R. dominica* adults are extensively captured and studied in the landscapes surrounding grain storage, it is still unclear how they are utilizing these landscapes. Thus, the

purpose of this research was to use population genetics and molecular gut content analysis to determine if populations of insects found in natural landscapes interact with those infesting nearby storage structures (Objective 1) and to determine their resource utilization in these habitats (Objective 2).

To accomplish these goals, *R. dominica* adults were trapped from three locations in Manhattan, KS: the Konza Prairie, the Kansas State University Feed Mill, and the MKC Co-op during the months of July-September in 2017, May-September in 2018, and April-August in 2019. These locations represent a native tallgrass prairie, a small volume grain facility that processes grain harvested from nearby fields, and a large commercial-scale elevator that buys and sells grain from over 24 of the 101 counties in Kansas. Comparisons of the population structures of insects caught at each location over a 3-year period were used to determine gene flow between locations. Specifically, this analysis allowed determination of whether *R. dominica* migrating through native landscapes can infest food storage structures or, conversely, whether *R. dominica* found in native landscapes of the Konza Prairie originate from storage structures, and perhaps are attempting to migrate to new food sources when population levels in storage structures rise.

For the molecular gut content study, *R. dominica* was trapped in two different tallgrass prairies (Konza Prairie, Manhattan, KS and Nine Mile Prairie, Lincoln, NE) from June to October in 2020, which correlates with their peak flight activity. Amplicon sequencing of plant-specific PCR primers were used to identify food resources used by *R. dominica* and ascertain how resource utilization changed from summer to fall. Confirming the consumption of natural resources is expected to improve insights into how *R. dominica* interacts with food resource in alternate habitats a Such new information is pivotal to implementing improved integrated pest

management (IPM) strategies to reduce or prevent the immigration of insects from the landscape into storage structures and food processing facilities.

Chapter 2 - Population dynamics of lesser grain borer in Manhattan, KS

Abstract

The lesser gain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae) is a cosmopolitan stored product pest that can be found infesting raw, dried grains and finished commodities. Although they are most commonly found infesting man-made structures used to house stored grains and food products, they can also often be captured in natural landscapes, such as prairies. However, it is not known if individuals from these other agricultural landscapes interact with populations that infest nearby storage facilities. To address this knowledge gap, *R. dominica* populations were surveyed at three field sites throughout peak flight seasons in 2017-2019, including a large-scale grain elevator, a research flour mill, and a native tallgrass prairie. The use of double restriction enzyme digestions followed by DNA sequencing was used to generate 20,635 markers to compare population structures at the three field sites throughout the three-year period. Our results show that there were four prominent genetic clusters (K = 4) identified from beetles collected at all three locations between 2017 and 2018. Heavy admixture of beetles caught in these locations was also observed between these field seasons. In contrast, results from 2019 highlight significant differences in genetic structure from previous years, especially in the tallgrass prairie, which is likely attributed to the high amount of rainfall observed during the trapping season and the lower amounts of individuals caught during this field season relative to 2017-2018. These findings can be used in conjunction with preventative pest management systems to reduce the spread of lesser grain borer.

Introduction

Collectively, feeding damage from stored product insects accounts for approximately 10% of damage to dried, stored commodities after harvest worldwide, with losses reaching up to 20% in developing countries (Phillips and Throne 2010). Damage is usually the direct result of insect feeding as the presence of frass, exuviae, and other byproducts of insect activity typically render commodities unsuitable for human consumption. Additionally, insect activity generates moisture that creates a niche for fungal and microbe growth (Atanda 2011). The global population is projected to reach over 9 billion people by 2050. As the climate becomes more variable and extreme weather events become more common, improving the durability of raw grains and finished food products in storage and protecting them from insect colonization are imperative for ensuring global food security.

Stored product insects are most commonly found in man-made structures for bulk grain storage and food processing (Phillips and Throne 2010). Once insects infest these structures, they persist, and their populations can expand rapidly because they have access to abundant food resources. Fumigations with phosphine are routinely used to control stored product insects, but resistance is becoming more common, and population levels occasionally rebound (Campbell et al. 2010, Buckman et al. 2013). Although the exact source(s) of the insects that recolonize these structures after fumigations is ambiguous, insects that persist in small spillage piles outside these facilities or insects from adjacent landscapes are potential culprits (Campbell and Arbogast 2004). Supporting this hypothesis, stored product insects are routinely observed in tallgrass prairies, woodlands, and agricultural fields (Campbell et al. 2006, Semeao et al. 2013, Holloway et al. 2020).

One stored product insect commonly observed in natural landscapes is the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae) (Collins et al. 2017, Wakil et al. 2021).

Although *R. dominica* is known as a primary pest of wheat, rice, sorghum, corn, and other high-valued stored commodities it can feed, reproduce, and complete the development on acorns from several different oak species and it can potentially feed on seeds from wild grasses (Edde 2012, Zeeshan Majeed et al. 2015, Daglish et al. 2017),. *Rhyzopertha dominica* also responds behaviorally to volatiles from plants native to tallgrass prairies and woodlands (Ching'oma et al. 2006, Mahroof et al. 2010, Edde 2012). These prior studies suggest that *R. dominica*, along with other species of stored product insects, may utilize alternate food resources in these natural landscapes. Notably, the family Bostrichidae contains many species of wood-boring beetles, and *R. dominica* adults are capable of boring into twigs in laboratory studies (Edde et al. 2006, Jia et al. 2008); however, this behavior has not been observed in wild populations, and it is unclear how exactly they use these natural habitats (Mahroof et al. 2010).

One possibility is that these insects are dispersing through these landscapes in search of suitable food resources. These insects are strong fliers (Dowdy 1994, Ridley et al. 2016, Holloway et al. 2020), and flight activity is often observed outside grain storage facilities (Leos-Martinez et al. 1986, Dowdy and McGaughey 1994, Toews et al. 2006). In conjunction, *R. dominica* have an increased tendency to fly when food resources are diminished or quality declines, overcrowding occurs, and during increased periods of warm temperatures (Dowdy and McGaughey 1994, Perez-Mendoza et al. 1998, 1999, Edde and Phillips 2006). Flight behavior is also more common in gravid females that have recently mated, suggesting that females may leave heavily infested structures to seek suitable food resources for their offspring (Ridley et al. 2016).

Although dispersal has been previously documented in *R. dominica*, only a handful of studies have examined the population dynamics and structure of *R. dominica*. Corderio et al. (2019) studied the population structure of *R. dominica* across nine states in the United States

(AR, CA, KS, LA, ND, OK, SC, TX, and WA). Overall, a high degree of admixture was observed among all populations regardless of geographical distance, and the volume of grain received was a significant driver of dispersal, suggesting that human-aided movement via the transportation of infested grain was an important factor in explaining population structure. Additionally, higher genetic diversity was observed in populations collected from the Great Plains region, where grain is stored in high volumes, suggesting this region serves as a source population for the rest of the United States. In an earlier study, a similar lack of genetic differentiation was observed in seven populations collected from four different counties in Kansas (Chen et al., 2015). However, the authors proposed long-range dispersal as an explanation for this admixture. Likewise, high genetic homogeneity was observed in *R. dominica* sampled over 7,000 km^2 in South Queensland, Australia (Guedes et al. 1997, Ridley et al. 2016). The authors concluded that long-range dispersal was primarily responsible for the lack of population structure as the anthropogenic movement of infested grains between adjacent farms is not common in this region.

In contrast, McCulloch et al. (2020) observed much higher genetic diversity and more significant population structuring in Turkey compared to what has been observed in the United States and Australia. The lower degree of admixture was attributed to Turkey's more localized agricultural practices and the more mountainous landscape, which likely restricts dispersal. These population studies collectively suggest that a myriad of factors influences the distribution of *R. dominica* and that these factors can have different influences on population structure over different spatial scales.

One question that was not addressed with these previous studies is whether insect populations found in natural landscapes interact with those found in nearby storage structures. Thus, our current objective was to study the population structure and dynamics of *R. dominica* in

the USA in three neighboring habitats over three years. Specifically, the three locations selected for this study are within 10 miles of each other near Manhattan, Kansas, USA. They include a tallgrass prairie, a small-scale flour mill, and a large grain co-op that receives grain from over 24 counties in Kansas. Trapping was also performed for three consecutive years at all locations to determine how gene flow changed over time. Observing local interactions between insects found in natural landscapes and nearby food processing facilities can provide insight into whether *R. dominica* moving through these landscapes could serve as source populations for nearby storage structures. These results can formulate integrated pest management (IPM) strategies to manage landscapes surrounding grain storage and prevent or reduce infestations.

Materials and Methods

Trapping *R. dominica*

Rhyzopertha dominica were trapped over three years from 2017 to 2019. The locations selected for this study included different habitats around Manhattan, KS, where *R. dominica* are routinely observed, including the Konza Prairie Biological Station (39.1069 N, 96.6091 W), which is a native tallgrass prairie (hereafter referred to as Konza), the Kansas State University Hal Ross Flour Mill (39.2061 N, -96.5906 W), which is a small scale flour mill that processes grains collected from nearby fields on the Kansas State University Agronomy Farm (hereafter referred to as flour mill), and a grain co-op located in southeastern Manhattan, KS (hereafter referred to co-op), which maintains a large grain elevator and stores and sells grains from over 24 counties in the state.

Trapping was conducted during peak flight season, which spans from April to October in Kansas, but can vary depending on temperature and humidity (Edde et al. 2006). Trapping timeframes for each field season were refined by examining trends in trap catches from the previous year. In 2017, trapping commenced in July and ran through October until there were no

more insects caught. In 2018, traps were set out earlier in May due to the high numbers of insects caught in Konza Prairie in July during the previous field season. As in 2017, trapping concluded in October when no more insects were caught. In 2019, trapping began in April in response to a high number of *R. dominica* caught in May in Konza Prairie during the 2018 field season and concluded in October. This timeframe was adjusted to catch individuals moving through Konza Prairie earlier in the flight season.

For all trapping *R. dominica*, Lindgren funnel traps were baited with a synthetic aggregation pheromone lure (Trece Inc., Adair, OK, product no. 3158-25) and 50 grams of organic whole wheat. Six Lindgren traps were placed biweekly at each of the three field sites and collected after two days. Insects were sieved from the grain and stored at -80ºC until DNA extraction. Insects collected were then identified using a key specific to Bostrichidae to ensure that only *R. dominica* were represented within the study (Sites et al. 2011).

Selection of *R. dominica* for Library Preparation

With thousands of insects captured over three years, we focused our initial analysis on 384 individuals to determine: 1) how populations from each location changed over the three flight seasons, 2) how the populations from each location changed within a single flight season, and 3) whether there were any interactions between populations from the three locations within a single flight season or over multiple years. We divided the field season into three major time points and selected 16 insects from every combination of location x timepoint: early season (April – July), mid-season (July – September), and late-season (September – October).

ddRAD-Seq Library Construction

Genomic DNA was extracted from individual *R. dominica* adults with the Nucleomag DNA Isolation Kit (Macherey-Nagel, Duren, Germany) using the KingFisher Flex (Thermo-Fisher, Waltham, MA) following the manufacturer's protocol. DNA was eluted in 100 µL of

elution buffer, and yields were quantified on a Syngery HTX Multi-Mode Microplate Reader with a Take3 assay (Biotek, Winooski, VT). Reduced representation ddRAD-Seq libraries were used for population genetics analysis. First, the program ddradseq-tools was used to perform *in silico* restriction digestions on the assembled genome of *R. dominica* (NCBI Bioproject PRJNA449115) with the rsitesearch.py script to facilitate the selection of appropriate restriction enzyme combinations (Mora-Márquez et al. 2017). Over 100 different combinations of restriction enzymes were tested, and combinations that generated the largest numbers of fragments between 175 and 500 bp were prioritized for testing in the lab. The restriction endonucleases NlaIII and MluCI (New England Biolabs, Ipswich, MA) generated high concentrations of DNA fragments within the desired range and were selected for use.

Briefly, 250 ng of DNA from each insect was digested using two units each of NlaIII and MluCl in a total reaction volume of 50 µL for 30 minutes at 37 °C. Successful digestion was confirmed via gel electrophoresis at 100 V for one hour with a 1.5% agarose gel in 1X TBE buffer. Subsequent adapter design, ligation, and size selection followed the recommendations of Peterson et al. (2012). After digestion, P1 adapters with barcodes were ligated to the digested DNA using 80 units of T4 DNA ligase (New England Biolabs, Ipswich, MA) at 22 °C for 2.5 hours in 40 µL reaction volumes. DNA > 100 bp in length was purified using 1.5X volume AMPure beads (Beckman Coulter, Loveland, CO). Samples were then pooled to form eight libraries containing DNA from 48 individual *R. dominica*, and a broad size selection between 175 to 325 bp was performed on a Blue Pippin (Sage Science, Beverly, MA) using a 1.5% gel cassette (Product Number: BDF1510).

Following size selection, PCR was performed in 20 µL reaction volumes to add the barcoded P2 adapters to the samples. Each reaction contained 20 ng of DNA, 4 µL Phusion HF Buffer, 1 µL forward primer (10 µM), 1 µL dNTPs (4 µM), 1 µL reverse primer (10 µM), and

0.2 µL Phusion DNA polymerase (New England Biolabs, Ipswich, MA). Thermocycling conditions consisted of an initial 30s denaturation at 98°C followed by 10 cycles of 98°C for 15s, 55°C for 30s, and 72°C for 20s, and a final extension of 72°C for 30s. Libraries were again purified using AMPure beads (Beckman Coulter, Loveland, CO) to remove residual primer-dimers. The library quality was checked on the 4150 TapeStation System (Agilent, Santa Clara, CA) using the D1000 high sensitivity assay and yields were quantified using the DNA High Sensitivity Assay on a Qubit fluorometer (Thermo-Fisher, Waltham, MA). The eight libraries were combined in equimolar ratios into a single pool for sequencing on four lanes of an Illumina HiSeq4000 with 150 x 150 paired-end reads (Novogene, Sacramento, CA). Read depths of approximately 3 to 5 million reads per sample were achieved.

Quality Filtering and Genotyping

The raw reads were analyzed with FastQC ((Andrews, 2010) to assess the quality and adapter content before further processing. The libraries were demultiplexed and residual rad adapters were removed using the process_radtags script in Stacks v1.44 (Catchen et al. 2013) and cutadapt (Martin, 2011), respectively. Subsequently, the program cutadapt to cull any residual adapters from the reads. We used default parameters except that the parameter n was set to five to allow multiple adapters to be trimmed from reads and reads less than 75 bp after trimming were discarded. The remaining high-quality reads were quality checked again with FastQC and mapped to the genome assembly of *R. dominica* using the bwa aligner. The ref_map.pl script of the Stacks pipeline was used to assemble mapped reads into loci, remove PCR duplicates, remove mapped reads with insert lengths > 500 bp, and phase heterozygous loci for downstream analysis. Subsequently, the Stacks population module was used to filter the loci to retain high confidence loci and alleles for population analysis. Minimal stack depth was set to two, and only loci that occurred in at least 60% of the individuals within each location and were represented in

all three areas were retained for analysis. Additionally, the maximum observed heterozygosity allowable was set to 0.5 to avoid potential paralogous loci, and a minor allele frequency of 0.05 or higher was required.

Genetic Diversity and Defining Population Clusters

Genetic diversity statistics for each time point and location were generated based on the retained SNPs using the population module in Stacks. Observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (FIS), and nucleotide diversity (π) across the genome were estimated. FST values were also calculated for each combination of year and sampling locations at the early, middle, and late timepoints. The program STRUCTURE v2.3.4 was used to identify the most likely number of unique genetic clusters in the entire dataset (Pritchard et al. 2000). STRUCTURE results for K values of 1-20 with 750,000 reps and a burn-in of 500,000 were compiled and summarized using the program Structure Harvester to obtain the likelihood of each K values using the Evanno method (Earl and vonHoldt, 2011). Clumpak was used to visualize the genetic clusters across each combination of sampling location x year x timepoint using Markov clustering algorithm computed by the CLUMPP software (Kopelman et al., 2015).

Analysis of Molecular Variance (AMOVA) and the Discriminant Analysis of Principal Components (DAPC), was performed to detect population differentiation using SNP markers using 1999 permutations using the poppr package in R (Kamvar et al., 2015).

Results

SNP and genotype discovery

Across the eight library pools, 2,142,830 loci were built from matching forward and reverse reads. After filtering, 58.1% of reads and 1,850,915 loci were retained with a mean number of sites per locus being 121.4 with the effective per-sample coverage mean being 1.0x.

The population module of Stacks was set to a minimum depth of two and to retain loci that were present in 60% of all individuals which filtered the number of loci to 20,635 for analysis.

Nucleotide and genetic diversity

Overall, the observed heterozygosity (H_0) for each location was lower than the expected heterozygosity (H_E), and across all locations there was also a high inbreeding coefficient (F_{IS}), indicating that there are less heterozygotes than predicted (Table 1). When observing all samples in the data, H_0 was 0.0486 ± 0.0014 and H_E was 0.2656 ± 0.0010 with an inbreeding coefficient of 0.8331 ± 0.1428.

A two-way ANOVA analysis was performed followed by a Tukey's HSD test to observe if these statistics were influenced by location, year, or interaction between these two variables. Observed heterozygosity (H0) was lower in 2019 than in 2018 and 2017 ($F_{2,22} = 7.14$, $P < 0.01$) across all locations. The expected heterozygosity was significantly lower in the Konza Prairie and the flour mill in 2019 ($F_{2,22} = 11.54$, $P < 0.05$) compared to the co-op and other year comparisons. Inbreeding coefficients were lower in the Konza Prairie in 2019 and in the flour mill in 2019 and 2019 ($F_{2,22} = 3,70$, $P < 0.05$). Konza Prairie and the flour mill in 2019 also had a lower nucleotide diversity ($F_{2,22} = 3.79$, $P < 0.05$) compared to previous years.

FST is a pairwise measurement for genetic variance and its values range from 0 to 1, with FST = 1 implying a high degree of differentiation and 0 implying no genetic differentiation. FST across all samples ranged from the lowest at 0.0254 to the highest at 0.5477 when considering each location by time point and year, with the mean being 0.0690 for the pairwise analysis (Figure 1)).

In 2017, the FST calculated for each timepoint and location over the three years ranged from 0.0337 to 0.0506 (mean FST = 0.0431 ± 0.003), with individuals sampled from the flour mill in the early field season being the most divergent overall (mean FST = 0.0473 ± 0.001). In

contrast, insects collected from the co-op late in the field season were the least divergent overall during this field season (mean FST = 0.0372 ± 0.0008). In 2018, FST values ranged from 0.0284 to 0.1147 with the overall mean FST value being 0.0560. During this field season, the population observed in the flour mill during the late time point in the field season was the most divergent from the other locations throughout the year (mean FST = 0.0675 ± 0.0075) and Konza early was the least divergent (mean FST = 0.0344 ± 0.002). In 2019, the FST values ranged from 0.0542 to 0.5477 with a mean of 0.0982. The most divergent population was the flour mill Early (mean FST = 0.2569 ± 0.063) and the least divergent was co-op Late (mean FST = 0.07917 ± 0.0065).

Table 1: Genetic diversity statistics from Konza, flour mill, and co-op locations across three years with 384 individuals and 20,635 loci from ddRADseq data for variant nucleotide positions. Observed heterozygosity (H_0), expected heterozygosity (H_E), nucleotide diversity (π), and inbreeding coefficient (FIS) were calculated between each location, year, and timepoint combination.

Location	Time	Year	H0	HE	π	FIS
Konza	Early	2017	0.042±0.001	0.224±0.001	0.244±0.001	0.522±0.009
Konza	Early	2018	0.041±0.001	0.172±0.002	0.207±0.002	0.319±0.006
Konza	Early	2019	0.041±0.001	0.237±0.001	0.25±0.001	0.614±0.012
Konza	Mid	2017	0.041±0.001	0.235±0.001	0.249±0.001	0.607±0.011
Konza	Mid	2018	0.041±0.001	0.236±0.001	0.25±0.001	0.615±0.012
Konza	Mid	2019	0.042±0.001	0.249±0.001	0.256±0.001	0.745±0.018
Konza	Late	2017	0.042±0.001	0.24±0.001	0.252±0.001	0.645±0.013
Konza	Late	2018	0.042±0.001	0.245±0.001	0.254±0.001	0.694±0.013
Konza	Late	2019	0.042±0.001	0.216±0.001	0.238±0.002	0.488±0.009
Flour Mill	Early	2017	0.042±0.001	0.248±0.001	0.257±0.001	0.71±0.015
Flour Mill	Early	2018	0.041±0.001	0.24±0.001	0.253±0.001	0.642±0.014
Flour Mill	Early	2019	0.041±0.001	0.248±0.001	0.257±0.001	0.703±0.014
Flour Mill	Mid	2017	0.041±0.001	0.243±0.001	0.254±0.001	0.675±0.012
Flour Mill	Mid	2018	0.042±0.001	0.244±0.001	0.255±0.001	0.663±0.013
Flour Mill	Mid	2019	0.042±0.001	0.242±0.001	0.254±0.001	0.659±0.01
Flour Mill	Late	2017	0.042±0.001	0.241±0.001	0.253±0.001	0.65±0.01
Flour Mill	Late	2018	0.042±0.001	0.242±0.001	0.25±0.001	0.689±0.016
Flour Mill	Late	2019	0.042±0.001	0.238±0.001	0.251±0.001	0.619±0.008
Co-op	Early	2017	0.042±0.001	0.241±0.001	0.254±0.001	0.63±0.012
Co-op	Early	2018	0.041±0.001	0.243±0.001	0.253±0.001	0.683±0.011
Co-op	Early	2019	0.042±0.001	0.243±0.001	0.253±0.001	0.681±0.012
Co-op	Mid	2017	0.041±0.001	0.25±0.001	0.258±0.001	0.728±0.016

Co-op	Mid	2018	0.041±0.001	0.193±0.001	0.226±0.002	0.38±0.006
Co-op	Mid	2019	0.04±0.001	0.102±0.001	0.141±0.002	0.153±0.003
Co-op	Late	2017	0.041±0.001	0.059±0.001	0.087±0.002	0.072±0.004
Co-op	Late	2018	0.041±0.001	0.134±0.002	0.172±0.002	0.225±0.005
Co-op	Late	2019	0.041±0.002	0.063±0.001	0.093±0.002	0.078±0.004

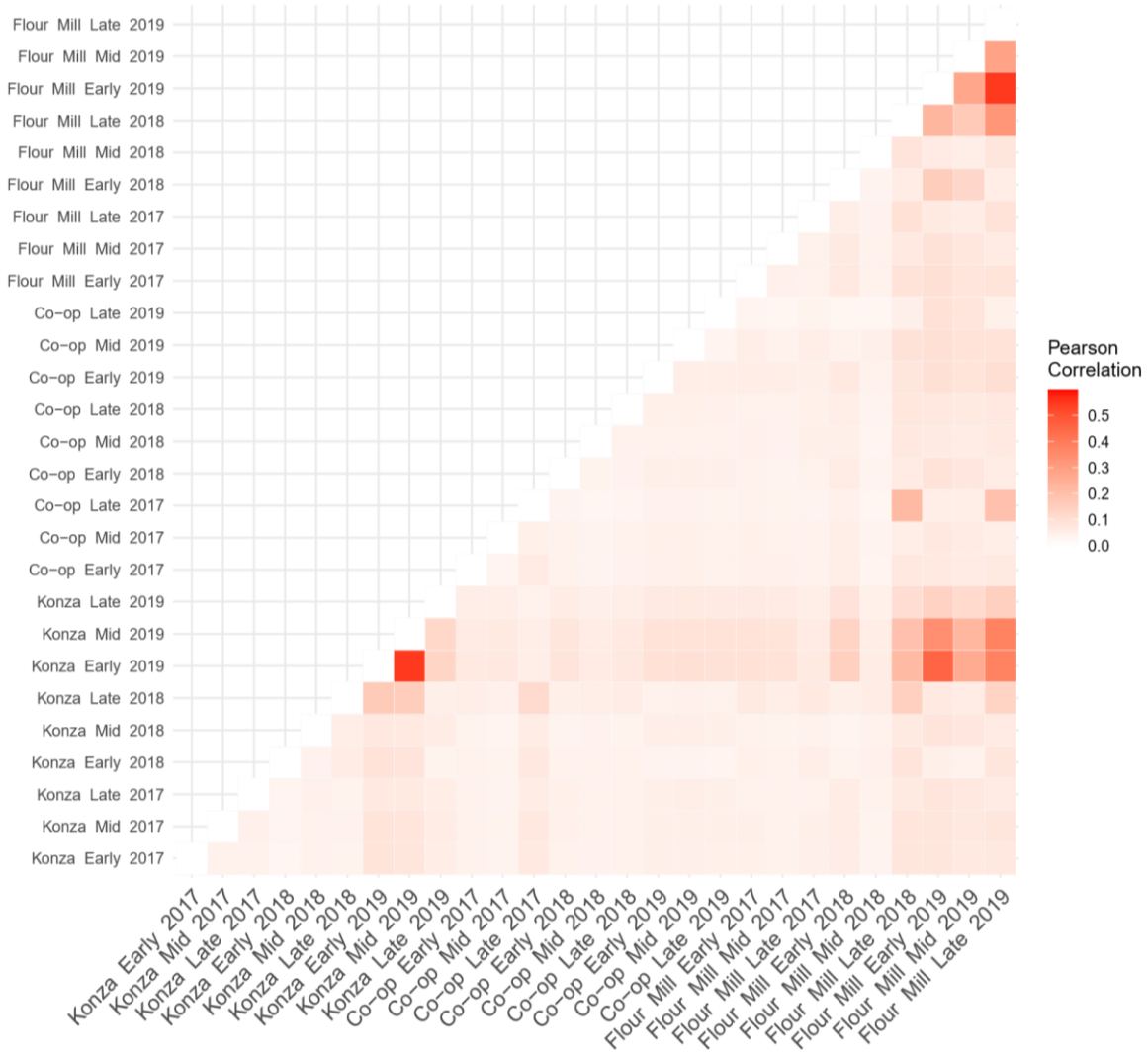

Figure 1: Heatmap of calculated pairwise FST means from Stacks v1.44 using 20,635 loci across all locations, timepoints, and years.

Population structure and clustering

AMOVA analysis between the location and years showed that variation between populations within a year accounted for 0.25% (P = 0.001, Table 2) and variation within samples account for 21.01% (P = 0.001) of total variation. The highest amount of variation across all samples was

found between samples within a location which accounted for 78.74% of the total variation (P = 0.001). Variance between years had a negative variance (σ = -0.064), which can essentially be interpreted as 0, while variation between locations within a year had a sigma value of 7.63. This result indicates that none of the variance in the data is explained by year and a very small portion of the overall variation is explained by location.

Structure results indicate that four is the most likely number of distinct genetic cluster through the Evanno method (K = 4, Figure 2). Observation of K = 4 shows relative abundance of genetic clusters does not change over time for the locations, aside from the flour mill in early and mid in 2017 where there is a higher abundance of cluster 2 and flour mill late in 2017 where the most prominent genetic cluster is 3. The DAPC analysis shows significant admixture when considering both location and year, especially in Konza and the co-op in 2017 and 2018 (Figure 3). Significant admixture was also observed between the locations in the 2019 field season, but less overall genetic variation was detected in that field season, potentially due to lower trap captures. In addition, Konza and the flour mill in 2019 were more dissimilar to the rest of the locations and timepoints and had significantly less variation, probably due to the lower number of individuals caught at those locations during the 2019 field season. Interestingly, significant differentiation was detected between the individuals collected in the flour mill in 2017 and the rest of the locations and years.

Table 2: AMOVA results across all samples with sigma representing variance and significant P values bolded.

Source of variation	d.f.	SS	Mean Sq.	Sigma (σ)	% of Variation	P value
Variation Between Years	2	12085.56	6042.78	-0.0641	-0.0021	0.326
Variations Between Locations Within Year	6	35979.82	5996.64	7.63	0.2535	**0.001**

Variation Between Samples Within Locations	375	2015296.62	5372.12	2370.79	78.74	**0.001**
Variation Within Samples	384	242893.8	632.54	632.54	21.01	**0.001**
Total	767	2306255.79	3006.85	3010.901	100	

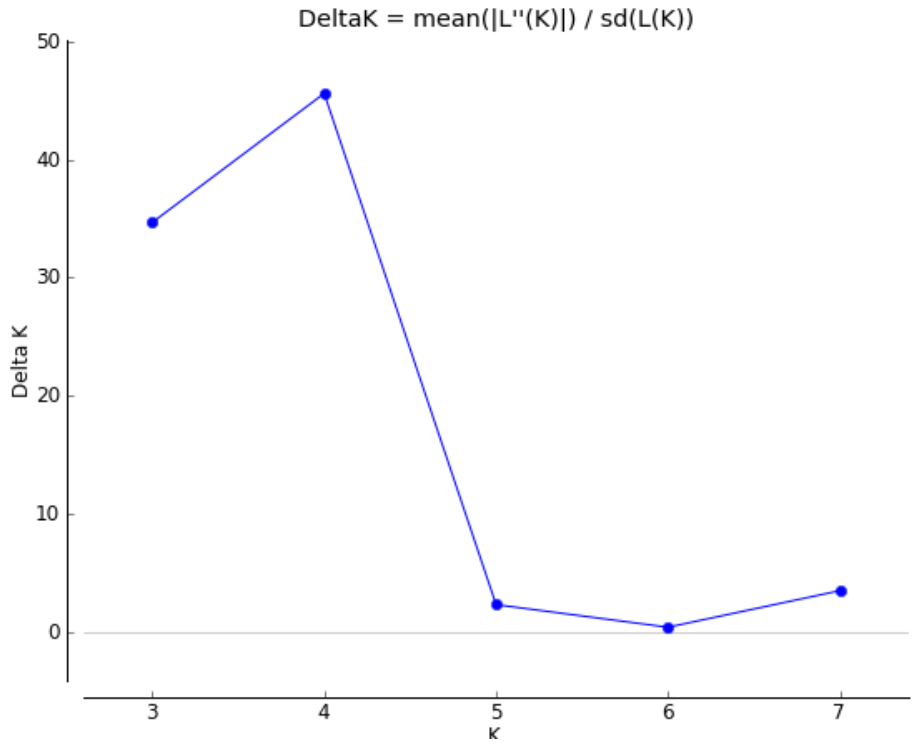

Figure 2: STRUCTURE HARVESTER results showing DeltaK = 4 using the Evanno method for best population structure.

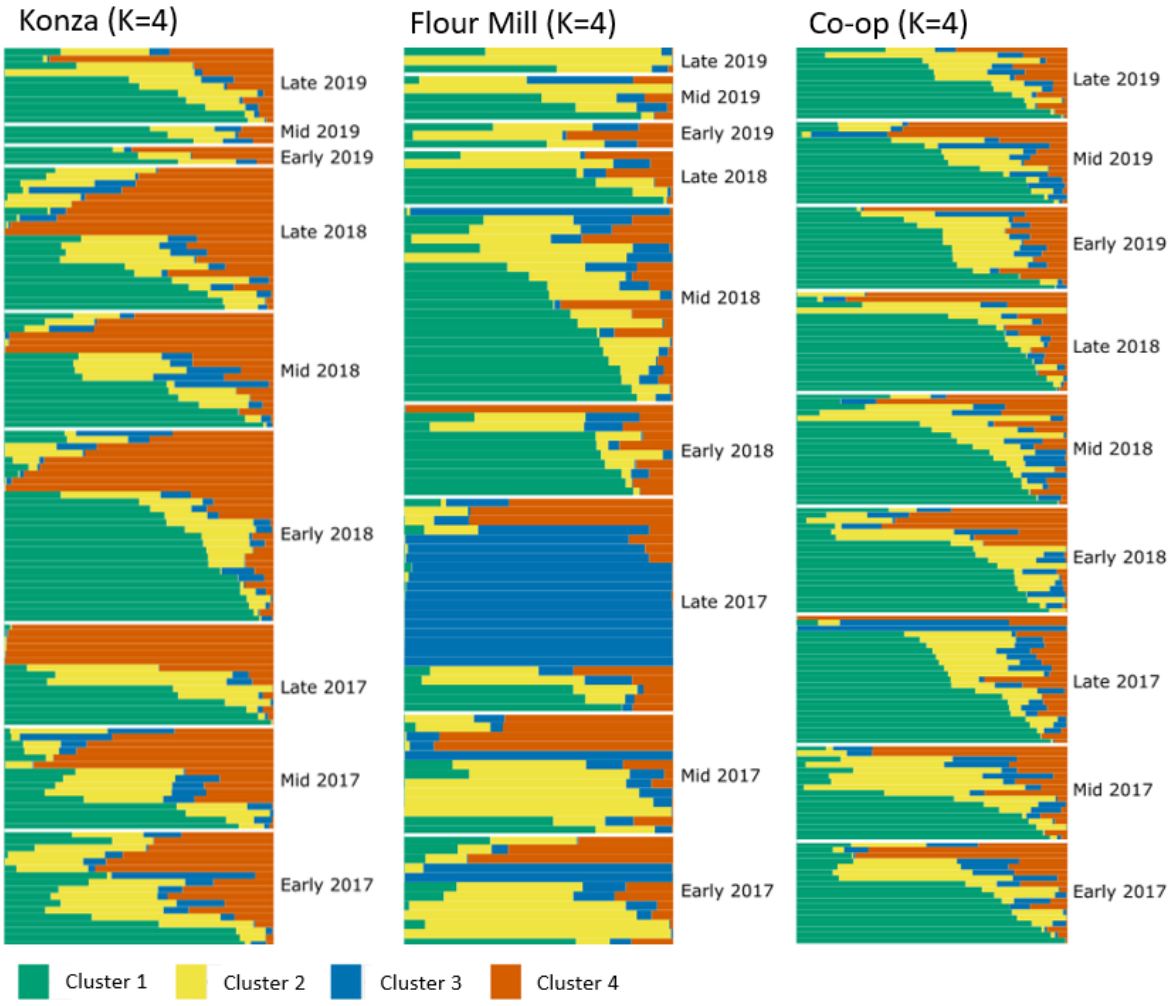

Figure 3: Distruct plot of K = 4 grouping of individual genotypes in each location and time point.

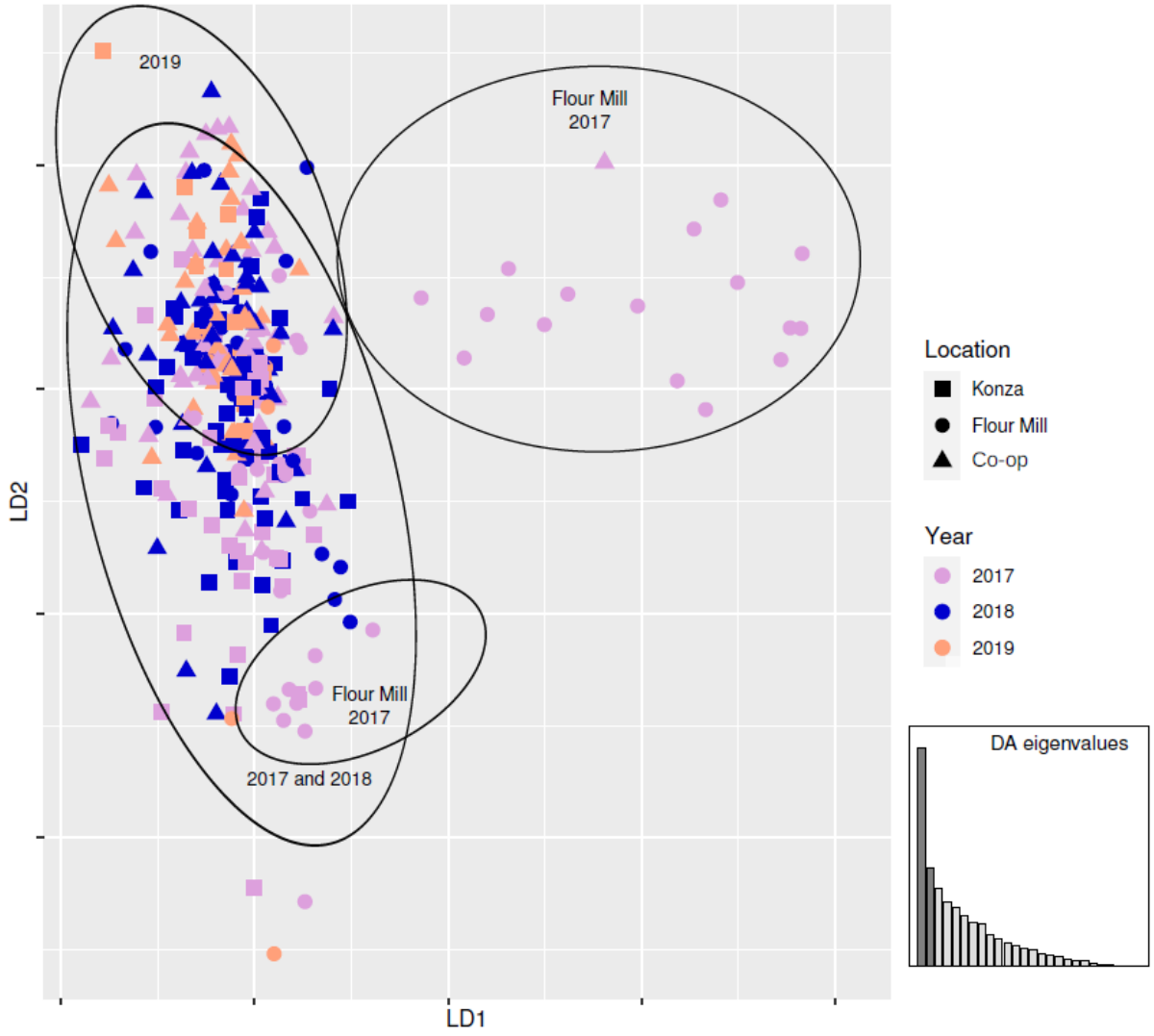

Figure 3: Discriminant analysis of principle components analysis discriminating the three locations across 2017, 2018, and 2019. Individual points represent one *R. dominica*. Groupings highlight trends seen in the analysis.

Discussion

Spatiotemporal impacts on genetic variance

Genetic variation within each of the three locations was not significantly different between 2017 and 2018. The absence of variation across a time scale may be due to the lack of environmental change that bulk stored commodities are subjected to and makes divergence by selection unlikely due to the food resources and shelter available to them (Drury et al., 2009;

Phillips and Throne, 2010). With the lack of external environmental pressure and the physical shelter that grain storage provides, *R. dominica* populations could persist year to year with little disturbance in grain mill and co-op settings. This is especially the case with the flour mill, since it is used as a research facility and does not store bulk amounts of grain, however, would have the most sensitivity to incoming sources of infested grain due to the low number of individuals caught at this location. 2019 was variable from 2017 and 2018, which is likely due to the high amount of rainfall during this time period and the low number of individuals captured.

The abundance of *R. dominica* caught in the native prairie area with little genetic variation between each year may be indicative of overwintering in nearby grain storage when outside temperatures are low (Edde, 2012; Jia et al., 2008; V F Wright et al., 1990). This phenomenon could explain the admixture observed between the co-op elevator and Konza over multiple field seasons and the genetic similarity of individuals caught on the co-op over a three-year time period, as well as the high number of individuals found within the co-op across the trapping season. When observing FST, for Konza 2017, individuals in were more genetically similar than 2018 and 2019, with 2019 being the most divergent, suggesting that over time their populations could be impacted by immigration and other environmental factors and creating more heterogenous populations. When observing FST, for Konza the population sampled in 2019 was most divergent from the other two field seasons, suggesting that over time, populations could be impacted by immigration and other environmental factors and creating more heterogenous populations. In addition, although the co-op elevator could be the major source for insects collected in Konza, individuals could persist in this location for long periods of time where they may interact with other immigrating individuals from other storage structures. This scenario is possible as 34 species of trees, six species of grasses, and thirteen species of forbs and

shrubs have been reported to be alterative food or breeding sources of *R. dominica*, with many of them being local or native to Kansas (Edde, 2012).

More longitudinal sampling at these three locations over a more extended period of time could help identify additional environmental factors that contribute to population dynamics of *R. dominica* in Manhattan, KS. With additional analysis of further years, it could help determine if populations have predictable genetic changes with a shift in population as beetles migrate through the landscape, or if they are the same persisting population and the impacts of environmental conditions on their genetic structure.

Population structure

The most variant location of the three was the flour mill, especially in the year 2017. This may be due to the practices of mills compared to large grain co-ops and unmanaged natural landscapes. Grain mills in the United States tend to have a low tolerance for live insects and insect damaged kernels (IDK) compared to grain storage facilities (Edde, 2012; Flinn and Hagstrum, 2001). These standards have facilitated the option of more strict pest control methods, such as sanitation, to prevent and treat populations of *R. dominica*. Variance could also be attributed to a potential import of infested grain in late 2017, as colonization of stored product pests can occur from the movement of infested sources of grain. *Rhyzopertha dominica* also requires whole grain for development of larvae to adults, and since the flour mill processes these whole grains down, it limits their persistence in these types of facilities.

Though there was variance in the grain mill compared to both the co-op and prairie landscape, heavy admixture and geneflow were observed between the locations and across the three years. This is supported by the low observed heterozygosity compared to the expected heterozygosity and high inbreeding coefficients. The high geneflow can be attributed to the dispersal behavior of *R. dominica*. Fecund females have a high tendency to fly, and in addition,

these beetles can fly long distances (Cogburn et al., 1984; Dowdy, 1994; Edde et al., 2006). A previous genetic study on populations of *R. dominica* found that Kansas was one of the most heterogenous populations compared to ten other states, likely due to the high amount of grain movement (Cordeiro et al., 2019).

Conclusion

This study has shown local interactions of populations of *R. dominica* caught in a prairie, co-op, and flour mill. The three areas sampled represent a variety of locations where these insects can be found. Though they are called the lesser grain borer and are notably found infesting human structures, they have been collected in prairie areas in high volumes, and with the lack of genetic variation between the prairie caught and co-op and flour mill beetles imply that are able to move freely between the grain structures and natural landscapes. Grain insects may be able to sustain populations after fumigation and treatment of structures by taking refuge in natural prairies or migrate to grain storage as temperatures in the outside environment change, causing the admixture observed across the populations. By observing these small-scale interactions, we have a better understanding of how these populations behave and have more informed IPM strategies to prevent insects from entering grain from natural landscapes.

Chapter 3 - Resource utilization of the lesser grain borer in the Konza Prairie and Nine Mile Prairie

Introduction:

Stored product insects are well-known for exploiting raw and finished commodities and can cause significant economic damage post-harvest. Common habitats for these insects include food processing and storage facilities where they can feed on raw grains and other high-value commodities (Phillips and Throne 2010). Though they are commonly found in anthropomorphic structures, some species are found in other landscapes, including the members of the family Bostrichidae (Mahroof et al. 2010, Edde 2012). This taxonomic family contains many wood-boring powder post beetles (subfamily Lyctinae) and two major stored product insects (subfamily Dinordeinae), the lesser grain borer, *Rhyzopertha dominica*, and the larger grain borer, *Prostephanus truncatus*. *Rhyzopertha dominica* is a cosmopolitan pest that primarily attacks stored grain but can also exploit a variety of other food sources such as nuts, dried fruits, and other dry stored goods (Edde 2012, Zeeshan Majeed et al. 2015). These insects are common in temperate and tropical locations worldwide (Zeeshan Majeed et al. 2015, Daglish et al. 2017) and represent primary pests whose larvae feed internally on the whole, undamaged kernels of grains of wheat, sorghum, corn, and rice. Though *R. dominica* is a predominantly pest of stored products, it is often found in other landscapes, such as agricultural fields, woodlands, and tallgrass prairies (Ching'oma et al. 2006). However, it is currently unknown how the insect uses these alternate landscapes.

One possible explanation for the presence of *R. dominica* in these landscapes is that they may serve as habitat or resource patches as insects search for suitable food and oviposition sources. While gravid females can lay eggs within the same food cache from which they emerge, *R. dominica* often initiate flight when populations are high or when food quality declines (Perez-

Mendoza et al. 1999). *Rhyzopertha dominica* have been well documented flying outside of grain storage (Daglish et al. 2017, Jian 2019). Mark-recapture studies have detected mean flight distances ranging from 260 m (Mahroof et al. 2010) up to 1000 m away from a release point depending on the habitat (Ching'oma et al. 2006, Daglish et al. 2017, Holloway et al. 2020). A second explanation for this phenomenon is that resident populations of *R. dominica* exist in these woodland and prairie landscapes.

Additionally, Ridley et al. (2016) documented genetically homogenous populations of *R. dominica* located over 100 km apart. Without a precise mechanism for anthropogenic movement between the locations studied, the authors proposed that long-range dispersal was primarily responsible for the high levels of admixture observed. Although it is unlikely that *R. dominica* populations could survive year-round in locations where temperatures reach below freezing, it is possible for them to persist year-round in these habitats in the southern United States (Edde 2012) and migrate to more northern locations during the spring and summer months. Despite this possibility, it is currently unknown whether resident populations exist in these landscapes or if beetles caught here originated from nearby human structures or potentially migrated from the southern United States (Jia et al. 2008, Mahroof et al. 2010).

Woodland habitats and tallgrass prairies do not contain known food hosts for *R. dominica*; however, these insects could exploit alternative food sources as they traverse through these habitats. Laboratory studies have been conducted to determine if they can feed and reproduce on various plant materials under laboratory conditions (Wright et al. 1990, Edde and Phillips 2006, Jia et al. 2008). In the earliest study, Wright et al., 1990 evaluated the suitability of several types of fruits and seeds found in Kansas for feeding and oviposition under laboratory conditions. Insects could survive for more than one month on fruits of sandhill plum, and progeny emerged on several species of tree acorns collected from the field, indicating that these

substrates were suitable for both adult and larval feeding and development. Likewise, Edde & Phillps (2006) demonstrated that *R. dominica* could also produce viable progeny on acorns and that they also were attracted to volatiles from cedar, pine, and acorns.

Furthermore, both male and female *R. dominica* were captured in the field in traps baited with acorn volatiles, but only when the traps were also baited with *R. dominica* pheromone. Finally, Jia et al. (2008) also investigated the host suitability of various resources found in Kansas prairie landscapes, including twigs, acorns, and seeds from forbs and grasses. Although insects would readily feed on all these resources, survival was generally lower relative to wheat. Furthermore, *R. dominica* could reproduce on acorns from six different *Quercus* spp., but only if they were previously damaged. Although these studies highlight the suitability of alternate food sources for *R. dominica* in prairie landscapes, all the studies were conducted under laboratory conditions, and it is unknown if they use any of these resources as food or refugia in the field. Furthermore, the emergence of *R. dominica* from acorns in the field has not yet been observed.

Although laboratory studies are valuable for assessing host suitability for insect feeding and reproduction (Hepler et al. 2021), they are inherently limited in scope and generally focus on assessing the suitability of a small number of prominent species present in landscapes where insects have been caught or observed (Mahroof et al. 2010, Whitaker et al. 2019, Evans and Kitson 2020). Given the diversity of plant species and food resources that an insect could potentially interact within many habitats and landscapes, it is very likely that not all potential hosts have been assessed for suitability, especially for smaller insect species whose behavior is difficult to observe in the field like *R. dominica*. Gut content analysis of field-caught insects can provide additional insights into resource utilization without the need to directly observe feeding behavior (Matheson et al. 2008, Zhu et al. 2019). For example, staining of gut contents collected from a close relative of *R. dominica*, *P. truncatus* (Bostrichidae), revealed the presence of both

starch and lignin in field-caught insects from several agroecosystems in Benin (Borgemeister et al. 1998). This finding indicates that these insects had recently fed on both amylaceous substrates and lignified plant materials, such as grain and woody stems, respectively. Although this finding confirmed that *P. truncatus* exploits alternative food resources, the specific plant species consumed were not identified.

DNA metabarcoding analyses commonly make use of polymerase chain reaction (PCR) followed by next-generation sequencing to amplify and sequence barcoding regions of DNA from food consumed by insects for taxonomic classification. This method is now a popular approach used to study feeding interactions in a variety of phytophagous insects, although it has also been used to identify gut contents of insects belonging to other feeding guilds (Derocles et al. 2014, Macías-Hernández et al. 2018, Kitson et al. 2019, Macgregor et al. 2019). Chloroplast-specific genes are common targets of DNA metabarcoding studies of the gut contents of insect herbivores. Common markers used in these studies include *trnL* (Cooper et al., 2016) and *rbcL* (Matheson et al. 2008, Wallinger et al. 2013). Although both markers have been successfully used to study insect-plant interactions, previous studies have shown that the *rbcL* gene can be used to identify gut contents from a range of insects that feed on woody plants and grasses and that products from these insects have high sequence similarity to sequences from known plant taxa that have been archived in NCBI (Matheson et al. 2008, Avanesyan and Lamp 2020).

The purpose of this study was to use DNA metabarcoding analysis with plant-specific primers to characterize the gut contents of *R. dominica* caught in prairie landscapes. For this approach, insects were trapped in two different tallgrass prairie locations in Nebraska and Kansas from June through September 2020 for DNA metabarcoding analysis with primers targeting the *rbcL* locus. Molecular identification of gut contents will allow us to determine if insects are utilizing food resources in these prairie habitats or simply exploiting grain caches from nearby

storage structures and how resource utilization in these landscapes' changes over the field season.

Materials and methods

Trapping *R. dominica* in tallgrass prairies

Rhyzopertha dominica were trapped in the Konza Prairie in Manhattan, KS (39.1069° N, 96.6091° W) and the Nine Mile Prairie in Lincoln, NE (38.9122° N, 91.7344° W) from June through October in 2020. Six Scentry® Delta sticky traps (Billings, MO) were deployed six feet apart at each location on a weekly basis and baited with a synthetic aggregation pheromone lure (Trece Inc., Adair, OK, product number 3158-25).

Gut dissection

Insects were removed from the traps with forceps and stored in 95% molecular grade ethanol at -20ºC. Glue from the sticky traps was removed by washing the insects briefly with 100% acetone. Insects were subsequently rinsed with Milli-Q water to remove any residual acetone prior to dissection. Bostrichids collected from the traps were identified to species level using a key from The Bostrichidae (Coleoptera) of Missouri (Sites et al. 2011) and insects identified as *R. dominica* were retained for analysis. Whole guts were dissected from insects in a sterile saline solution containing 128 nM NaCl, 4.7 nM KCl, and 2.8 nM $CaCl_2$ (Zhu and Baker 1999). The entire alimentary canal, including the foregut, midgut, and hindgut, was removed from each insect by applying pressure to the abdomen and gently pulling off the insect's head.

DNA extraction of *R. dominica* guts

Each sample consisted of a pool of ten whole guts from *R. dominica* collected simultaneously within each location. In brief, each pool was placed into 500 µL Bead Bashing Buffer from the DNeasy Plant Pro Kit (Qiagen, Hilden, Germany) and homogenized using a Bullet Blender (Next Advance, Troy, NY) at maximum speed for 5 minutes. DNA was

quantified on the Qubit Fluorometer using the DNA Broad Range Assay (Invitrogen, Carlsbad, CA). If DNA concentrations were < 10 ng/μL, samples were reconcentrated using the DNA Clean and Concentrator kit to > 20 ng/uL (Zymo Research, Irving, CA).

Testing *rbcL* primers on *R. dominica* feeding on wheat

Although *rbcL* primers have been successfully used to assess the diet of phytophagous insects in the field, it was unclear if they would efficiently amplify DNA from ingested grain, whose chloroplast content is lower compared to the vegetative tissues of plants. *Rhyzopertha dominica* adults were reared on hard winter wheat at 27 °C, 65% relative humidity, and 14:10 L:D. The whole guts were dissected from these insects and DNA was extracted as described above. Fifty ng of DNA was amplified with the *rbcL* (Levin et al. 2003) (5'-ATGTCACCACAAACAGAGACTAAAGC-3') and *rbcLa*-R primer set (5'-GTAAAATCAAGTCCACCRCG-3') (Kress 2017) with the following thermal cycling conditions: initial denaturation at 95°C for 3 min followed by 35 cycles of 95°C at 30s, 60°C at 30s, and 72°C for 1 min, and a final extension of 72°C for 7 min. Reaction volumes were 25 uL consisting of 50 ng DNA, 0.25 uL ExTaq DNA polymerase (TaKaRa, Kyoto, Japan), and 0.4 μM of each primer. Gel electrophoresis on a 1.5% agarose gel was performed at 120V for 1.5 hrs to validate successful PCR amplification. Products were then treated with ExoSAP-IT (Thermo-Fisher, Waltham, MA) and sequenced bidirectionally on an 3730xl DNA Sequencer (Applied Biosystems, Waltham, MA) by Eton Biosciences (San Diego, CA).

Polymerase chain reaction to amplify plant-specific DNA and Illumina MiSeq Sequencing

To ensure that the purified DNA was free of any inhibitors and sufficient integrity for DNA metabarcoding, we first performed PCR reactions using insect-specific primers that targeted the mitochondrial COI gene using the following primer set: LCO1490 (5'-

GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) and the same reaction volumes as described for the *rbcL* primer set above. PCR thermal cycling conditions were: initial denaturation at 95°C for 2 min followed by 28 cycles of 95°C at 30s, 56°C at 30s, and 72°C for 1 min, and a final extension of 72°C for 7 min. Products were analyzed by gel electrophoresis as described above. Next, DNA samples that produced successful COI PCR products were amplified with *rbcL* primers that were modified to contain the Illumina P5 and P7 adapter sequences (underlined): *rbcL* (Levin et al. 2003) (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGTCACCACAAACAGAGACTAAAGC-3') and *rbcLa-R* (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTAAAATCAAGTCCACCRCG-3') (Kress 2017). The *rbcL* region was selected because it is recognized as one of the universal plant barcoding loci by the Consortium for the Barcode of Life (CBOL) and sequences derived from these regions can often be classified to the genus or even species levels with high degrees of confidence (Matheson et al. 2008, Hollingsworth et al. 2009, Wallinger et al. 2013). PCR reactions were performed in 25 uL volumes using ExTaq DNA polymerase (TaKaRa, Kyoto, Japan) as described above with 100 ng of input DNA. Thermal cycling conditions included an initial denaturation at 95°C for 3 min followed by 35 cycles of 95°C at 30s, 60°C at 30s, and 72°C for 1 min, and a final extension of 72°C for 7 min. Successful amplification was validated using gel electrophoresis as described above. The reactions were treated with ExoSAP-IT (Thermo-Fisher, Waltham, MA) following the manufacturer's protocol to remove excess primer-dimers. Products were subsequently barcoded with dual i5 and i7 barcodes using the Nextera XT DNA library preparation kit v2, index set A (Illumina, San Diego, CA) following the manufacturer's protocols. Reaction volumes were 50 uL and consisted of 50 ng *rbcL* PCR product, 0.25 uL Phusion High Fidelity Polymerase (New

England BioLabs, Ipswitch, MA), and 5 uL of each adapter. PCR reactions were performed as follows: initial denaturation at 72°C for 3 min followed by 8 cycles of 95°C at 10s, 55°C at 30s, and 72°C for 30s, and a final extension of 72°C for 5 min. Barcoded products were pooled together and sequenced to a depth of at least 100,000 reads per sample on an Illumina MiSeq instrument as 300 x 300 paired-end reads.

Data analysis of Miseq reads

The primary purpose of our study was to assess changes in diet over the entire field season at the two locations. To accomplish this, we divided the flight season into three major time points: early (June), middle (July), and late (August-September) and attempted to analyze at least three biological replicates at each time point at each location. However, due to low trap captures, it was not possible to achieve this level of replication at Nine Mile Prairie in the middle (n = 1) and early time points (n = 2), but results are included for qualitative comparisons. Reads were pre-processed to remove adapters and demultiplex the samples. Forward reads that did not contain identifiable primer sequences or had more than one mismatch with the assigned barcode were removed from the analysis using the USEARCH algorithm (Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010)(Edgar and Bateman, 2010). Forwards reads were denoised using the Divisive Amplicon Denoising Algorithm (DADA2) to minimize artifacts due to sequencing errors and remove PCR chimeras (Callahan et al. 2016). Reads were then assigned to operational taxonomic units (OTUs) at 97% nucleotide sequence similarity using UPARSE. After this stage, samples containing less than 500 total reads and singleton OTUs (e.g., OTUs that had only a single read assigned) were removed from the

dataset using the amptK filter command. Finally, taxonomy was assigned using the UTAX algorithm and the *rbcL* database (Edgar 2013, 2016, Bell et al. 2017).

Results

Taxonomic identification of plant DNA in insect guts

A total of 413 unique OTUs were identified but were subsequently reduced to 57 OTUs with 2,895,728 identified reads when OTUs that did not match to plant taxa (n = 7) or had no matches to known taxa were removed from the dataset (n = 105). In addition, OTUs that were assigned to the same species were consolidated into a single OTU. The remaining 57 OTUs were all classified as belonging to clade Viridoplantae, which includes all land plants and green algae. These OTUs were classified to 19 different orders with a majority of the OTUs assigned to Poales (26.3% of the OTUs), followed by Rosales (12.3%), Fabales and Fagales (8.8% each), Cupressales (7%), and others that comprised less than 3.5% of the total OTUs each. Furthermore, OTUs were classified into 22 unique families, with the most abundant family being Poaceae, which comprised 22.8% of the identified OTUs, and the next most abundant families being Fabaceae and Fagaceae, which each comprised 8.8% of the total OTUs. Of the 57 OTUs, 41 were identified to species level (Table 3).

Forty of the 57 unique OTUs were detected in guts collected in Konza prairie. In total, 38 of these OTUs could be conclusively classified to order, 37 to family, 32 to genus, and 27 to species. Of the 27 OTUs identified to species level, several were derived from grasses (n = 11), woody plants (n = 10), flowering plants (n = 3), herb (n = 1), and legumes (n = 2) were identified (Table 4). Notably, *Triticum aestivum* (bread wheat), *Glycine max* (soybean), and *Oryza sativa* (rice), which are known diets of *R. dominica*, were detected in insects caught in Konza Prairie; however, the relative abundance of reads derived from these taxa were lower compared to other taxa with 0.067%, 2.11%, and 0.039% of the reads being classified as *T. aestivum*, *G. max*, and

O. sativa, respectively. The other 23 species that were detected have not been previously confirmed as food sources for *R. dominica.*

Although fewer insects were trapped Nine Mile Prairie relative to Konza Prairie, 37 of the 57 OTUs identified in this study were detected in guts collected from this location. Overall, 35 OTUs could be assigned to order, 34 to family, 28 to genus, and 25 to species. Of the 25 species identified for this location, amplicons derived from grasses (n = 8) and legumes (n = 1) were also observed, along with woody plants (n = 12), and flowering plants (n = 4). Amplicons derived from *T. aestivum* (bread wheat), and *G. max* (soybean) were identified within these wild caught *R. dominica* and consisted of 0.031% and 1.94% of the reads respectively.

Additionally, we compared the OTUs detected in our analysis to the species that were tested for suitability for feeding and development in Edde 2012, which identified several grasses, forbs, shrubs, and trees as potential food and oviposition substrates for *R. dominica* (Edde, 2012). Collectively, nine of the families that were assessed in the Eddie 2012 study were also identified in our amplicon data, which included Annonaceae (custard apples), Cupressaceae (conifer family), Fabaceae (legumes), Fagaceae (beech, chestnut, oak family), Moraceae (mulberry family), Poaceae (grasses), Rosaceae (rose family), and Salicaceae (willow family). At the genus level, *Juniperus* (juniper), *Morus* (mulberry), *Prunus* (plum), and *Quercus* (beech) were identified in both studies and, at the species level, *Gleditsia triacanthos* (honey locust) was identified as a potential host in two different laboratory feeding studies (Jia et al., 2008; V. F. Wright et al., 1990)and our current study.

Relative abundance of OTUs

The relative abundance of each OTU was calculated for each location across the three timepoints by dividing the number of reads assigned to each OTU by the total number of reads that were derived from plant OTUs (Figure 4). Across Konza and Nine Mile Prairie, 2,207,600

and 688,128 reads derived from plant OTUs were identified, respectively. When considering both locations together, reads derived from OTUs assigned to the family Poacae were the most abundant (53.7%), followed by Fagaceae (13.6%), unidentified family (e.g., could not be assigned to family level, 11.1%), and Moraceae (6.7%). The unidentified family consisting of 11.1% of the population consisted of order Desmidiales (0.035%), the class Magnoliopsida (7.35%), and phylum Streptophyta (3.71%). In Konza Prairie, the relative abundance of amplicons derived from family Poaceae (55.8%) was highest. Other families detected that had a relative abundance of ≥ 1.0% included Fagaceae (17.9%), unidentified family consisting of members in the order Desmidiales and class Magnoliopsida (13.8%), Fabaceae (4.9%), Ranunculaceae (3.0%), and Cupressaceae (1.0%). In Nine Mile Prairie, the family Poaceae also had the highest relative abundance (46.7%) followed by Moraceae (28.2%), Cupressaceae (12.0%), Rosaceae (8.0%), order level identification of Magnoliopsida (2.3%), and Fabaceae (2.0%). The most abundant OTU that was detected in this study was identified as *Thinopryum ponticum.* It was prominent in both locations and across the entire field season. For example, in Konza, it comprised 38.1% of the total amplicons in the early season, 60.9% in the mid-season, and 44.6% in the late season. Similar trends were noted in Nine Mile Prairie with reads derived from this OTU comprising 38.1, 83.0, and 44.6% of the reads derived from early, mid, and late season, respectively.

Diversity and species richness

The Shannon diversity index is a metric that summaries diversity within a community by collectively accounting for richness (number of OTUs) and evenness (relative abundance of each OTU). In order to determine if the diversity of the diets differed between the two locations, we pooled the data from across the entire field season together and compared Shannon diversity metrics with an ANOVA. Overall, Shannon diversity did not differ between the two locations

($F_{1,18}$ = 1.692, P = 0.21, Figure 5). Nine Mile Prairie had an average Shannon diversity of 1.08 ± 0.17 (n = 6) while Shannon diversity in Konza Prairie was 0.81 ± 0.12 (n = 14). Moreover, Shannon diversity did not differ across the field season at either location (Konza: $F_{2,11}$ = 1.708, P = 0.226; Nine Mile Prairie: $F_{2,3}$ = 1.071, P = 0.446). Numerically, more OTUs were identified in the insects collected at Konza Prairie compared to Nine Mile Prairie; however, richness did not differ significantly did not between the two locations ($F_{1,18}$ = 2.114, P = 0.163). In Konza, 40 OTUs were identified in the early season, 35 in the mid-season, and 32 in the late season. In Nine Mile Prairie, 19 OTUs were identified in the early season, five in the mid-season, and 36 in the late season.

Across the sampling season, seven OTUs were detected in all three timepoints (OTU 1, 3, 4, 8, 9, 13, 24) from the early to the late season in Konza Prairie, four of which were grasses (OTU 1, 3, 9, 13) (Figure 6a). In Nine Mile Prairie, three OTUs were detected across the field season (OTU 3, 6, 13) with two being grasses (Figure 6b). Two OTUs identified as *Bromus erectus* (OTU 9) and an unidentified member of the family Poaceae (OTU 13) were detected in all Konza and Nine Mile Prairie samples. When comparing the same timepoint within the field season between both locations, the highest number of shared OTUS (n = 4) between the two locations OTUs (n = 4) was found in the late season. These OTUs were derived from two evergreen plants, one woody plant, and one grass (Figure 6c). When data from the full field season were pooled together, both locations shared 20 of the 57 OTUs (Figure 6d). These shared OTUs consisted of grasses (n = 7), woody plants (n = 7), flowering plants (n = 3), legume (n = 1), algae (n = 1), and land plant (n = 1).

Table 3: Taxonomic identification of OTUs identified as plant species used for analysis.

OTU#	Kingdom	Phylum	Class	Order	Family	Genus	Species
OTU1	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Cymbopogon*	*Cymbopogon jwarancusa*
OTU2	Viridiplantae	Streptophyta	sub Pinidae	Cupressales	*Cupressaceae*	*Juniperus*	*Juniperus coxii*
OTU3	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Thinopyrum*	*Thinopyrum ponticum*

OTU4	Viridiplantae	Streptophyta	Magnoliopsida				
OTU5	Viridiplantae	Streptophyta	sub rosids	Rosales	*Moraceae*	*Streblus*	*Streblus ascendens*
OTU6	Viridiplantae	Streptophyta	Magnoliopsida	Ranunculales	*Ranunculaceae*	*Delphinium*	*Delphinium cashmerianum*
OTU7	Viridiplantae	Streptophyta	sub Pinidae	Cupressales	*Cupressaceae*	*Hesperocyparis*	*Hesperocyparis bakeri*
OTU8	Viridiplantae	Streptophyta					
OTU9	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Bromus*	*Bromus erectus*
OTU10	Viridiplantae	Streptophyta	sub rosids	Fabales	*Fabaceae*	*Gleditsia*	*Gleditsia sinensis*
OTU11	Viridiplantae	Streptophyta	sub rosids	Fagales	*Fagaceae*	*Quercus*	
OTU12	Viridiplantae	Streptophyta	Magnoliopsida	Poales	*Cyperaceae*	*Cyperus*	*Cyperus rotundus*
OTU13	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*		
OTU14	Viridiplantae	Streptophyta	sub rosids	Rosales	*Moraceae*	*Morus*	
OTU15	Viridiplantae	Streptophyta	sub asterids	Lamiales	*Lamiaceae*	*Ocimum*	*Ocimum tenuiflorum*
OTU16	Viridiplantae	Streptophyta	sub rosids	Rosales	*Moraceae*	*Morus*	*Morus alba*
OTU17	Viridiplantae	Streptophyta	sub rosids	Crossosomatales	*Geissolomataceae*	*Geissoloma*	*Geissoloma marginatum*
OTU18	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Bromus*	
OTU19	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Triticum*	*Triticum aestivum*
OTU20	Viridiplantae	Streptophyta	sub rosids	Fagales	*Fagaceae*	*Castanopsis*	*Castanopsis sieboldii*
OTU21	Viridiplantae	Streptophyta	sub rosids	Rosales	*Rosaceae*	*Prunus*	
OTU22	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	-	*Poaceae sp. A.Guadamuz*
OTU23	Viridiplantae	Streptophyta	Zygnemophyceae	Desmidiales			
OTU24	Viridiplantae	Streptophyta	sub rosids	Fagales	*Fagaceae*		
OTU25	Viridiplantae	Streptophyta	sub rosids	Rosales	*Rosaceae*	*Prunus*	*Prunus caroliniana*
OTU26	Viridiplantae	Streptophyta	sub Pinidae	Cupressales	*Cupressaceae*	*Juniperus*	*Juniperus indica*
OTU27	Viridiplantae	Streptophyta	Magnoliopsida	Alismatales	*Hydrocharitaceae*		
OTU28	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Alopecurus*	*Alopecurus geniculatus*
OTU29	Viridiplantae	Streptophyta	-	Magnoliales	*Annonaceae*	*Fenerivia*	*Fenerivia humbertii*
OTU30	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Pogonatherum*	*Pogonatherum sp. OP*
OTU31	Viridiplantae	Streptophyta	Magnoliopsida	Solanales	*Convolvulaceae*	*Cuscuta*	*Cuscuta victoriana*
OTU32	Viridiplantae	Streptophyta	sub rosids	Malpighiales	*Salicaceae*	*Populus*	*Populus deltoides*
OTU33	Viridiplantae	Streptophyta	sub rosids	Fabales	*Fabaceae*	*Gleditsia*	*Gleditsia triacanthos*
OTU34	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Oryza*	*Oryza sativa*
OTU35	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	*Hypericaceae*		
OTU36	Viridiplantae	Streptophyta	-	Laurales	*Lauraceae*		
OTU37	Viridiplantae	Streptophyta	sub rosids	Fabales	*Fabaceae*	*Glycine*	*Glycine max*
OTU38	Viridiplantae	Streptophyta	sub rosids	Fabales	*Fabaceae*	*Glycine*	*Glycine tabacina*
OTU39	Viridiplantae	Streptophyta	sub asterids	Lamiales	*Pedaliaceae*	*Sesamum*	*Sesamum indicum*
OTU40	Viridiplantae	Streptophyta	-	Laurales	*Lauraceae*	*Lindera*	*Lindera sp. FU-*
OTU41	Viridiplantae	Streptophyta	Magnoliopsida	Asterales	*Asteraceae*	*Echinops*	*Echinops ritro*
OTU42	Viridiplantae	Streptophyta	-	Ranunculales	*Ranunculaceae*	*Hamadryas*	*Hamadryas magellanica*
OTU43	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Australopyrum*	*Australopyrum velutinum*
OTU44	Viridiplantae	Streptophyta	sub rosids	Rosales	*Moraceae*		
OTU45	Viridiplantae	Streptophyta	Polypodiopsida	Polypodiales	*Polypodiaceae*	*Goniophlebium*	*Goniophlebium persicifolium*
OTU46	Viridiplantae	Streptophyta	sub rosids	Rosales	*Rosaceae*	*Prunus*	*Prunus africana*

OTU47	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Amphibromus*	*Amphibromus fluitans*
OTU48	Viridiplantae	Streptophyta	-	Santalales	*Loranthaceae*	*Agelanthus*	*Agelanthus sansibarensis*
OTU49	Viridiplantae	Streptophyta	sub Cycadidae	Cycadales	*Zamiaceae*	*Encephalartos*	*Encephalartos ngoyanus*
OTU50	Viridiplantae	Streptophyta	-	Caryophyllales	*Amaranthaceae*	*Amaranthus*	*Amaranthus tricolor*
OTU51	Viridiplantae	Streptophyta	Liliopsida	Poales	*Cyperaceae*	*Schoenoplectus*	*Schoenoplectus triqueter*
OTU52	Viridiplantae	Streptophyta	Liliopsida	Poales	*Poaceae*	*Koeleria*	*Koeleria sp. Forest*
OTU53	Viridiplantae	Streptophyta	sub rosids	Fagales	*Fagaceae*	*Quercus*	*Quercus guajavifolia*
OTU54	Viridiplantae	Streptophyta	sub rosids	Fagales	*Fagaceae*	*Castanopsis*	
OTU55	Viridiplantae	Streptophyta	sub Pinidae	Cupressales	*Cupressaceae*		
OTU56	Viridiplantae	Streptophyta	Magnoliopsida	Solanales	*Convolvulaceae*	*Cuscuta*	*Cuscuta tinctoria*
OTU57	Viridiplantae	Streptophyta	sub rosids	Fabales	*Fabaceae*	*Gleditsia*	

Table 4: OTU identification subset into plant groups. Relative abundance was calculated for each location at the different trapping points.

Taxa/ Species (OTU)	Konza Early (N = 5)	Konza Mid (N = 5)	Konza Late (N= 4)	Nine Mile Early (N = 2)	Nine Mile Mid (N = 1)	Nine Mile Late (N = 3)
Grasses						
Alopecurus geniculatus (OTU28)	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%
Amphibromus fluitans (OTU47)	0.00%	0.00%	0.00%	0.05%	0.00%	0.00%
Australopyrum velutinum (OTU43)	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%
Bromus erectus (OTU9)	0.01%	0.02%	0.17%	6.52%	20.02%	9.98%
Cymbopogon jwarancusa (OTU1)	2.04%	0.58%	13.42%	8.45%	0.03%	0.00%
Koeleria sp. Forest (OTU52)	0.00%	0.00%	0.00%	0.05%	0.00%	0.00%
Oryza sativa (OTU34)	0.00%	0.10%	0.00%	0.00%	0.00%	0.00%
Poaceae sp. A. Guadamuz (OTU22)	0.00%	0.00%	7.22%	0.22%	0.00%	0.00%
Pogonatherum sp. OP (OTU30)	0.00%	0.00%	0.00%	0.02%	0.00%	0.00%
Populus deltoides (OTU32)	1.60%	0.00%	0.00%	0.00%	0.00%	0.00%
Thinopyrum ponticum (OTU3)	18.47%	60.88%	44.55%	28.39%	38.14%	83.02%
Triticum aestivum (OTU19)	0.00%	0.16%	0.02%	0.03%	0.01%	0.00%
Poaceae (OTU13)	11.23%	1.65%	2.45%	2.36%	2.23%	4.11%
Bromus (OTU18)	0.67%	0.00%	0.09%	0.63%	0.00%	2.87%
Cyperus rotundus (OTU12)	0.16%	0.01%	0.00%	0.00%	0.00%	0.00%
Schoenoplectus triqueter (OTU51)	0.48%	0.00%	0.00%	0.00%	0.00%	0.00%
Woody Plants						

Castanopsis sieboldii (OTU20)	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%
Encephalartos ngoyanus (OTU49)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Geissoloma marginatum (OTU17)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Hesperocyparis bakeri (OTU7)	0.00%	0.00%	0.06%	0.47%	0.00%	0.00%
Juniperus coxii (OTU2)	0.00%	0.00%	0.00%	0.03%	0.00%	0.00%
Juniperus indica (OTU26)	0.00%	0.00%	0.00%	0.02%	0.00%	0.00%
Lindera sp. FU- (OTU40)	0.00%	0.05%	0.00%	0.00%	0.00%	0.00%
Quercus guajavifolia (OTU53)	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%
Quercus (OTU11)	1.37%	0.00%	0.34%	0.00%	0.00%	0.00%
Fagaceae (OTU24)	24.72%	10.73%	18.43%	0.03%	0.12%	0.00%
Castanopsis (OTU54)	0.00%	0.00%	0.04%	0.00%	0.00%	0.00%
Cupressaceae (OTU55)	0.00%	0.00%	3.27%	11.50%	0.00%	0.00%
Prunus africana (OTU46)	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%
Prunus caroliniana (OTU25)	0.00%	0.00%	0.00%	0.10%	0.47%	0.00%
Morus (OTU14)	0.00%	0.00%	0.00%	4.67%	0.00%	0.00%
Prunus (OTU21)	0.00%	0.00%	0.00%	7.91%	36.51%	0.00%
Cuscuta victoriana (OTU31)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Gleditsia sinensis (OTU10)	0.00%	0.00%	0.63%	0.09%	0.00%	0.00%
Gleditsia triacanthos (OTU33)	0.00%	0.00%	0.00%	0.02%	0.00%	0.00%
Morus alba (OTU16)	0.00%	0.00%	0.00%	23.43%	0.00%	0.00%
Streblus ascendens (OTU5)	0.00%	0.00%	0.00%	0.04%	0.00%	0.00%
Moraceae (OTU44)	0.00%	0.00%	0.00%	0.08%	0.00%	0.00%
Gleditsia (OTU57)	0.00%	0.00%	7.81%	0.00%	0.00%	0.00%
Agelanthus sansibarensis (OTU48)	0.00%	2.14%	0.00%	0.00%	0.00%	0.00%
Fenerivia humbertii (OTU29)	0.00%	0.00%	0.11%	0.01%	0.02%	0.00%
Herb						
Ocimum tenuiflorum (OTU15)	6.24%	0.00%	0.00%	0.00%	0.00%	0.00%
Legume						
Glycine max (OTU37)	7.23%	0.00%	0.00%	1.94%	0.00%	0.00%
Glycine tabacina (OTU38)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Algae						
Desmidiales (OTU23)	0.14%	0.00%	0.02%	0.00%	0.01%	0.00%
Flowering Plant						
Amaranthus tricolor (OTU50)	0.00%	0.00%	0.00%	0.14%	0.00%	0.00%

Delphinium cashmerianum (OTU6)	0.00%	7.76%	0.22%	0.18%	0.29%	0.02%
Echinops ritro (OTU41)	0.00%	0.00%	0.00%	0.01%	0.04%	0.00%
Goniophlebium persicifolium (OTU45)	0.02%	0.00%	0.06%	0.00%	0.00%	0.00%
Hamadryas magellanica (OTU42)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Sesamum indicum (OTU39)	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%
Unidentified (OTU4)	24.45%	3.91%	1.07%	2.09%	2.06%	0.00%
Hydrocharitaceae (OTU27)	0.09%	0.00%	0.00%	0.00%	0.02%	0.00%
Hypericaceae (OTU35)	0.00%	0.00%	0.00%	0.33%	0.00%	0.00%
Lauraceae (OTU36)	0.00%	0.19%	0.00%	0.00%	0.00%	0.00%
Parasitic Plant						
Cuscuta tinctoria (OTU56)	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%

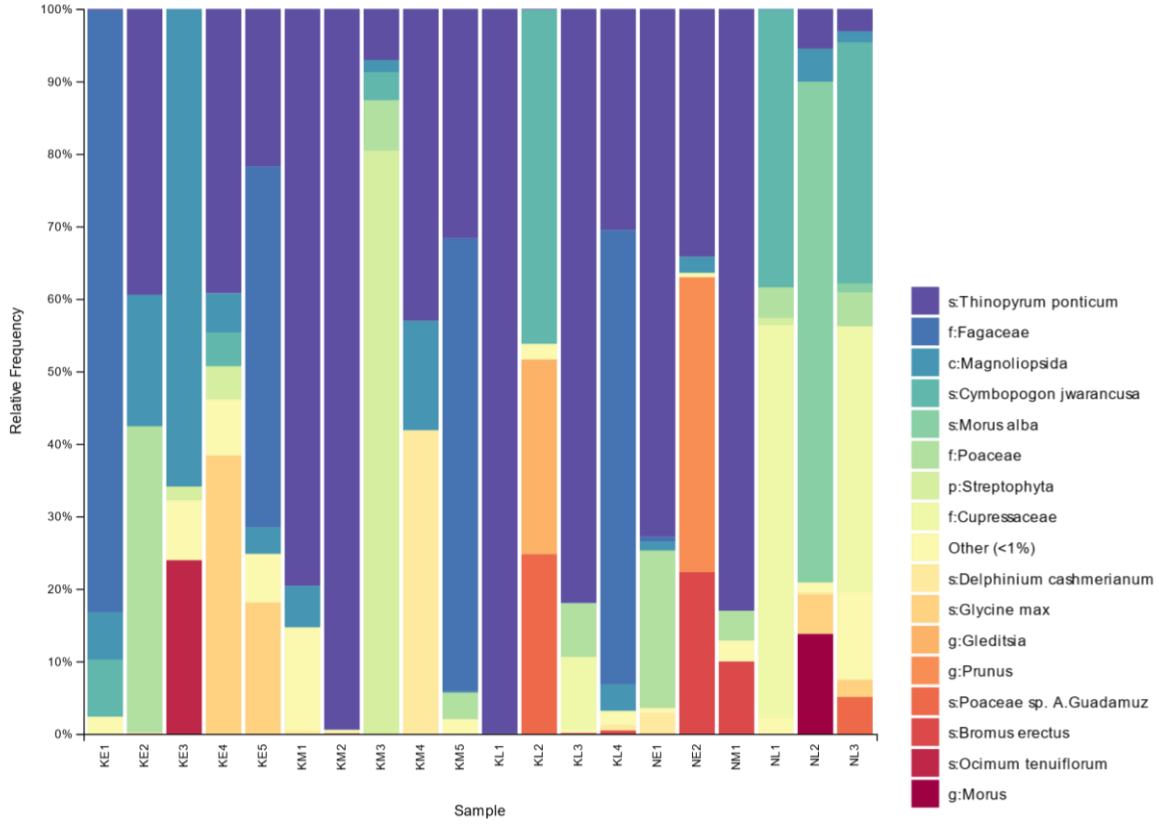

Figure 4: Relative frequency of OTUs for Konza and Nine Mile Prairie to species level of identification. Other category consists of all OTUs that composed of < 1% of the relative frequency of plant DNA detected.

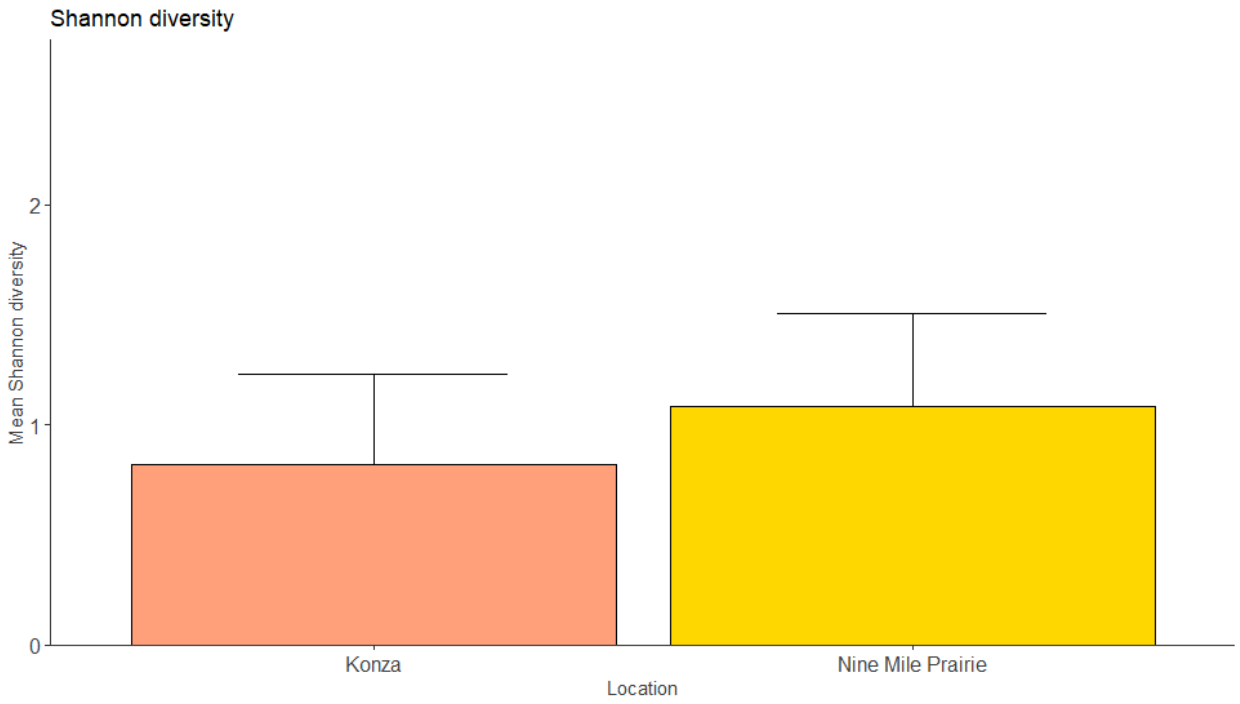

Figure 5: A Shannon Diversity metric for Konza and Nine Mile Prairie for the entire season.

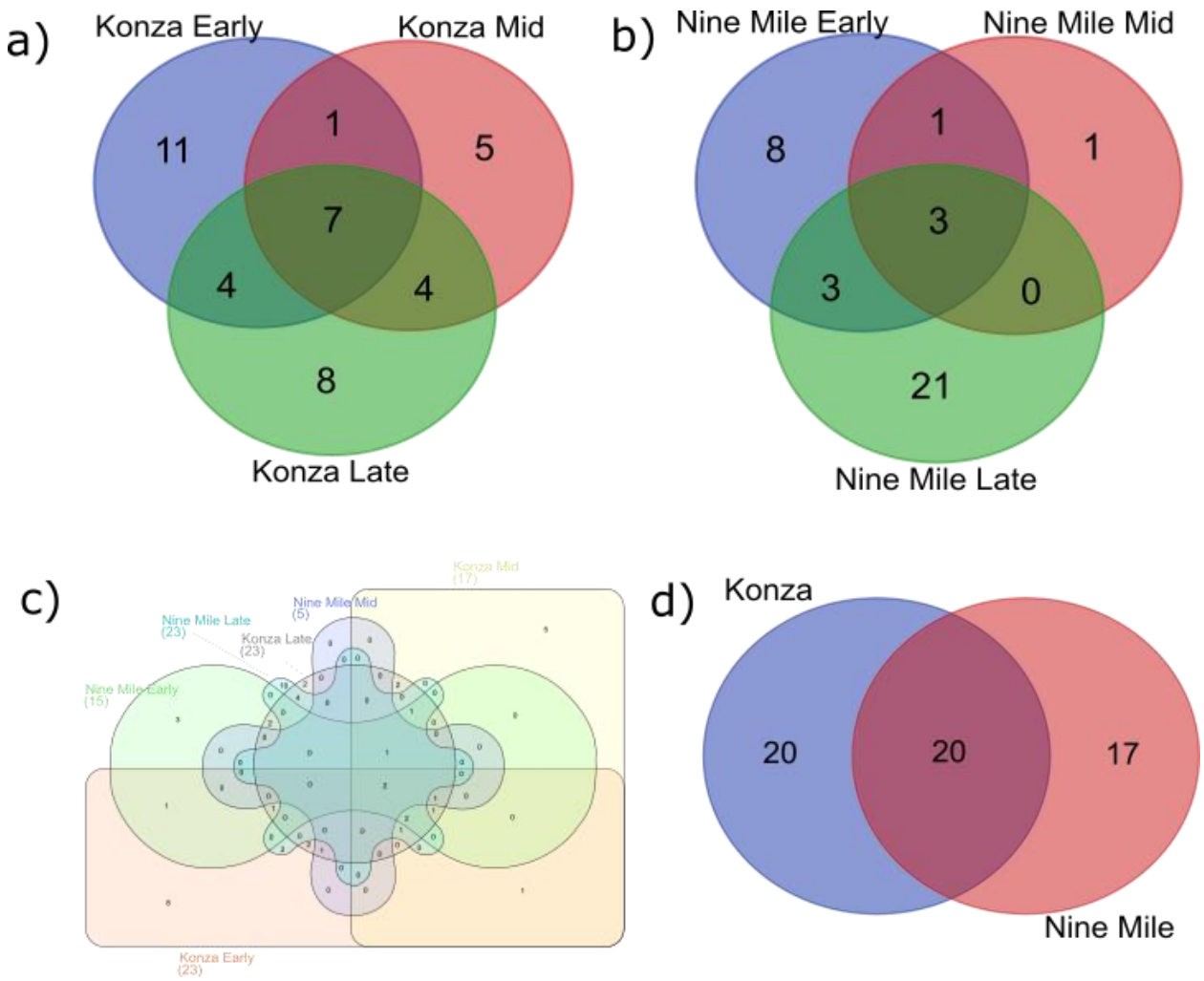

Figure 6: (a) Venn diagram comparison of OTUs found in Konza Prairie throughout the early, middle, and late trapping season, (b) Venn diagram comparison of OTUs found in the Nine Mile Prairie throughout the early, middle, and late trapping season, (c) Venn diagram comparison of OTUs found in Konza and Nine Mile Prairie across the early, middle, and late trapping season, (d) Venn diagram comparison of OTUs found in the Konza and Nine Mile Prairies.

Discussion

This study utilizes DNA metabarcoding to target the r*bcL* chloroplast gene within guts of *R. dominica* captured in prairie settings to determine how and if they are utilizing plant resources found in these agricultural landscapes. Although *R. dominica* is predominantly known as a primary pest of stored grains capable of feeding on undamaged kernels, its propensity to fly long distances and its presence in a variety of agricultural landscapes suggests that it may rely on

other food sources. Indeed, these insects can survive on acorns and other seeds found in prairie landscapes, but it is unknown if they use these resources in the field (Jia et al., 2008; V. F. Wright et al., 1990). Additionally, previous laboratory studies of *R. dominica* found that they were capable of feeding on several non-grain hosts for short periods of time (Edde and Phillips, 2006; Jia et al., 2008; Wright et al., 1990), although were unable to complete development while exclusively feeding on these materials. In this study, we used DNA metabarcoding analysis to assess gut contents of *R. dominica* caught in two different tallgrass prairies. We were consistently able to detect *rbcL* PCR products from plants in the guts of beetles caught in two different tallgrass prairies and we identified 57 OTUs that were classified to 41 different species of graminaceous and non-graminaceous plants.

DNA derived from common stored grains were detected in the gut contents of some insects captured in both Konza and Nine Mile Prairies, including soybean, rice, and wheat. Although soybean and wheat are among the major hosts documented for *R. dominica* and both crops are grown and stored in areas near these two tallgrass prairies, amplicons derived from these taxa were found in low abundance across the entire field season for both Konza (2.2%) and Nine Mile Prairies (2.0%). Although low in abundance across the entire field season, their abundance was slightly higher in the early season for Konza Prairie (7.3%) and in the late season for Nine Mile Prairie (3.4%). The most abundant OTU found in Konza (43.2%), and Nine Mile Prairies (28.4%) was derived from a graminaceous plant, *Thinospyrum ponticum.* This species is closely related to wheat and is often grown throughout the Great Plains region for grazing and hay production (Scheinost et al., 2008). Although not previously documented as a potential food source for *R. dominica*, fields where this crop is grown are located near both locations. Collectively, this finding suggests that some *R. dominica* found in prairie landscapes may be feeding on stored grain or crops nearby before entering this alternate habitat. In addition,

amplicons derived from other species found in both were also readily identified in guts collected from Konza and Nine Mile Prairie included G. *tricanthos* (honey locust), *M. alba* (mulberry), and *P. deltoides* (cottonwood), which suggests that *R. dominica* may utilize a broad range of food sources as they navigate through these landscapes (Kaul and Rolfsmeier, 1987; Nippert et al., 2019).

The diets of the Konza and Nine Mile Prairies were similar between locations and similar across the field season. In both locations, amplicons derived from members of the family Poaceae were the most abundant, which is the taxonomic family that includes *T. aestivum* and *T. ponticum*. Beyond these two plant species, other plant families that were detected at both locations included Annonaceae, Convolvulaceae, Cupressaceae, Fabaceae, Fagaceae, Geissolonataceae, Hydrocharitaceae, Ranunculaceae, and Rosaceae. Of these, only members of the family Fabaceae are grown for crop production and are often found in prairie habitats. In addition, there was no significant difference in diet composition throughout the field season, which suggests that these insects are utilizing similar resources in both habitats. This can be supported by the behavior of *R. dominica* as they can fly long distances and are prone to initiate flight if food resources decline (Cogburn et al., 1984; Dowdy, 1994; Edde et al., 2006; Perez-Mendoza et al., 1999). These insects have also had responses to non-grain host volatiles, which could lead them to prairie settings in search of a new food source (Edde and Phillips, 2006). Further research into the population dynamics of *R. dominica* is needed to determine the relationship between natural landscapes and grain sources.

One downside to our methodology is that multiple guts were pooled together in a single DNA extraction for analysis, which does not allow us to determine if individual beetles fed on multiple food sources as they transverse this landscape. This information would be relevant to know as it could provide some context as to whether these insects can feed on these hosts for

long periods of time or whether they are simply grazing on these resources when they encounter them to determine if they are suitable for longer-term feeding. The presence of multiple plant products within the guts of individual beetles could suggest that *R. dominica* grazes on multiple food hosts as it moves around until it finds suitable feeding and oviposition materials. Once an individual finds suitable food sources, it will release aggregation pheromones to attract other individuals to the area for increased mating opportunities. Additional feeding studies in the lab could elucidate whether these resources can support longer-term feeding and whether feeding would elicit the production of aggregation pheromones.

This information is also relevant because there have been many anecdotal reports of *R. dominica* feeding on and boring into wood. However, a recent detailed analysis of the *R. dominica* genome revealed that this insect does not code for any plant cell wall degrading enzymes that would enable it to extract nutrients from cellulose, hemicellulose, or any of the other major polysaccharides found in woody or vegetative tissues (Oppert et al., 2022). This finding is in agreement with several laboratory studies that showed low survival and poor development of insects that fed on twigs. Collectively, these previous findings indicate that woody tissues are probably not suitable substrates for feeding or development. Additionally, these previous findings suggest that *rbcL* amplicons derived from woody plants or deciduous tree species that were detected in *R. dominic*a in the current study were likely derived from fruits or seeds that can be digested by *R. dominica* or that beetles were simply grazing on twigs and other materials in search of more suitable feeding substrates.

Another key piece of data that would help inform and interpret the results of this study is understanding how often these insects need to feed in order to sustain flight activity and how long contents are retained in their guts after feeding. For example, we can infer that some insects collected in both prairies fed on stored grains, but the relative abundance of those products were

low relative to amplicons from other plant species indicating that they had fed more recently on food resources. Whether the insects can extract enough nutrients from the food items found in the two prairie landscapes to sustain flight activity in *R. dominica* is something that should also be investigated in the future.

Conclusion

The molecular identification of plant DNA inside the guts of prairie-caught *R. dominica* is an essential steppingstone to understanding the behavior and habitat utilization of these insects in alternate agricultural landscapes. An important finding from this study was the high abundance of amplicons derived from *T. ponticum* in insects caught in both locations, which has not been reported previously as a host for *R. dominica*. This species is grown throughout the Plains region for fodder for livestock. Although damage from *R. dominica* might not lead to major economic damages because this fodder is most commonly used as animal feed, it's possible that populations of insect that exploit this commodity could serve as source populations for infestation of nearby storage structures. Importantly, hay is frequently moved around between neighboring farms, which could provide additional means of dispersal for this insect on a more local scale. In previous population genetics studies, gene flow between *R. dominica* populations in neighboring storage structures was detected and attributed to dispersal by flight. However, the detection of *T. ponticum* in the gut suggests that movement of hay and other commodities between farms could also serve as an overlooked means of anthropogenic-mediated dispersal. Though they may use these species of grasses as energy sources as they migrate through the landscapes, their grains and seeds may be too small to support the development of *R. dominica* larvae. Taking into account the landscapes and fauna surrounding grain storage can aid in developing preventative methods of control to combat this destructive grain pest. Many of the prominent native flora that were detected were common trees (honey locust, mulberry, and

cottonwood) which can be found naturally in prairie landscapes and can serve as resources for *R. dominica*. Establishing physical barriers, such as insecticidal netting, between grain stores and areas that contain alterative hosts can reduce the risk of infestation. An extended survey of the gut contents of *R. dominica* in prairie areas along with molecular gut analysis of insects found in grain storage can develop a greater understanding of their resource utilization and further our knowledge of their behavior.

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