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*Tribolium castaneum*: A Model  
Insect for Fundamental and  
Applied Research

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**Keywords**

genetics, genomics, dispersal, olfaction, landscape ecology, insecticide resistance

**Abstract**

*Tribolium castaneum* has a long history as a model species in many distinct subject areas, but improved connections among the genetics, genomics, behavioral, ecological, and pest management fields are needed to fully realize this species' potential as a model. *Tribolium castaneum* was the first beetle whose genome was sequenced, and a new genome assembly and enhanced annotation, combined with readily available genomic research tools, have facilitated its increased use in a wide range of functional genomics research. Research into *T. castaneum*'s sensory systems, response to pheromones and kairomones, and patterns of movement and landscape utilization has improved our understanding of behavioral and ecological processes. *Tribolium castaneum* has also been a model in the development of pest monitoring and management tactics, including evaluation of insecticide resistance mechanisms. Application of functional genomics approaches to behavioral, ecological, and pest management research is in its infancy but offers a powerful tool that can link mechanism with function and facilitate exploitation of these relationships to better manage this important food pest.

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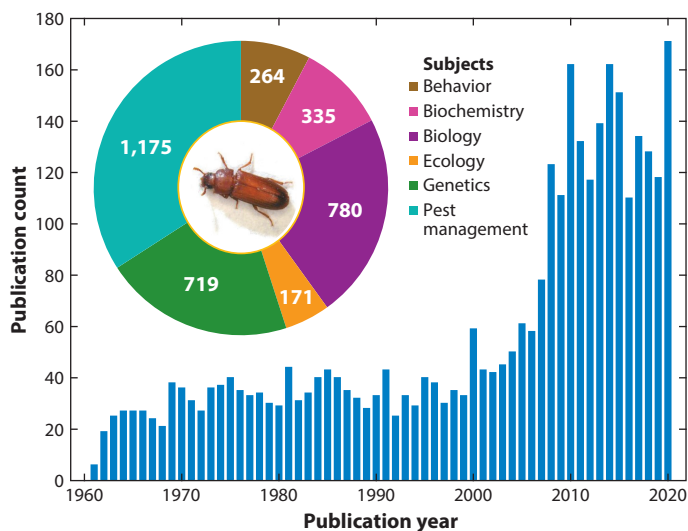
**Functional genomics:** a field attempting to understand the complex relationship between genotype and phenotype on a global (genome-wide) scale

## INTRODUCTION

*Tribolium castaneum* (Herbst 1797) (Coleoptera: Tenebrionidae), the red flour beetle, is an important international pest of stored products and has been extensively researched to improve pest management programs. This species also has long history as a useful model insect, and in recent years, its applications have been expanded (**Figure 1**). The 23 reviews of research between 1936 and 2021 show the scope of the research and the increasing importance of *T. castaneum* as a model insect species (**Table 1**). These reviews and **Figure 1** clearly show the evolution of *T. castaneum* as a model species in many subject areas, but there remains considerable untapped potential due to limited connections between the genetics and genomics research and its application to address behavioral and ecological questions and to improve pest management. In this review, we provide an overview of this species as a model and highlight some recent innovative research trends, specifically in areas where increased connections between genetics or functional genomics and behavior, ecology, and pest management research could be most impactful.

## IMPORTANCE AS A PEST

*Tribolium castaneum* is one of the most ubiquitous of the stored product insect pests, with a nearly worldwide distribution [reported in 156 countries (60; <https://www.cabi.org/isc/datasheet/54667>, [https://www.gbif.org/occurrence/search?offset=700&taxon\\_key=4990338](https://www.gbif.org/occurrence/search?offset=700&taxon_key=4990338))]. Its geographic distribution extends beyond locations with suitable climate (70) by exploiting environmentally controlled indoor environments and by frequent human-aided dispersal. This species is an especially important pest in wheat and rice mills but has a very broad host range and can be economically important throughout processed commodity distribution and storage systems



**Figure 1**

Annual publication counts (*histogram*) and research subjects of the publications (*pie graph*) on *Tribolium castaneum* in the past 60 years (1961–2020). A total of 3,444 publications were found from the Postharvest Document Service (now in Zotero; [https://www.zotero.org/groups/2423481/stored\\_product\\_pest\\_management/items](https://www.zotero.org/groups/2423481/stored_product_pest_management/items)) and PubMed (<https://pubmed.ncbi.nlm.nih.gov>) databases. These publications were categorized into six different subject areas: behavior, biochemistry, biology, ecology, genetics, and pest management. The numbers in the pie graph indicate the total publications in their respective subject areas.

**Table 1** List of past reviews using *Tribolium castaneum* as a model insect species (1936 to 2021)

Year(s)	Subject	Reference
1936	Biology	Good (55)
1962	Competition	Park (107)
1966	Genetics	Sokoloff (128)
1970	Spread of insecticide resistance	Dyte & Blackman (41)
1972	Population ecology	King & Dawson (76)
1972	Population ecology	Mertz (94)
1972, 1974, 1977	Behavior, biology, ecology, and physiology	Sokoloff (129)
1982	Genetic improvement in livestock and poultry	Bell (17)
1987	Aging	Soliman (130)
2008	Odorant receptors	Engsontia et al. (44)
2008	Developmental biology	Denell (36)
2008	Immunity to pathogens	Altincicek et al. (8)
2009	Developmental biology	Brown et al. (21)
2010	Olfaction and exoskeleton	Beeman et al. (14)
2011	Reproductive biology	Chandrasekar & Palli (31)
2012	Population ecology	Costantino & Desharnais (33)
2016	Immunity to pathogens	Milutinovic et al. (96)
2016	Epigenetics of host–parasite coevolution	Vilcinskas (140)
2018	Potential use of RNA interference	Kumar et al. (82)
2019	Biology, host range, and management	Abdullahi et al. (2)
2019	Physiology, biomedicine, and environment	Adamski et al. (3)
2020	Insecticide mode of action and mechanisms of resistance	Rösner et al. (119)
2021	Evolution and ecology	Pointer et al. (111)

(<https://storedproductinsects.com/new-species/comparison-of-species-list/>). The suitability of 215 commodities for development, reproduction, or population growth of *T. castaneum* has been studied (<https://storedproductinsects.com/biology/suitability-of-commodities-as-food-for-tribolium-castaneum/>), with reports of infestation published for 61 of these commodities (see the sidebar titled Human-Aided Global Movement of *Tribolium castaneum*).

*Tribolium castaneum* is an important pest because of its broad host range and its abilities to find and infest commodities and to rapidly increase in population size after establishment. *Tribolium castaneum* has one of the highest population growth rates of all stored product beetles due to its reproductive rate and long reproductive life (129), although cannibalism by adults and larvae can limit growth (7). Emigration from an infested commodity (59), dispersal through the landscape (25), and ability to find and infest dispersed commodities can result in rapid spreading of infestation throughout and between facilities (57, 116, 125). Direct damage can result from reductions in amount of commodity through feeding (<https://storedproductinsects.com/economic-losses-from-tribolium-castaneum-infestations/>). However, the greatest economic impact is due to

**Landscape:** an area of any scale that is spatially heterogeneous in at least one factor of interest

## HUMAN-AIDED GLOBAL MOVEMENT OF *TRIBOLIUM CASTANEUM*

Human-aided dispersal, such as in ships' cargos, has moved *T. castaneum* around the world. In all, 480 species of Coleoptera in 277 genera and 40 families were imported into England between 1957 and 1969; *T. castaneum* was the most often imported species every year and was also the most studied (6).

**Epigenetics:** heritable changes caused by modification of gene expression rather than alteration of the genetic code

**Satellite DNA:** highly repetitive DNA consisting of short sequences repeated a large number of times

**Pericentromeric heterochromatin:** a constitutive heterochromatin (highly condensed, tightly packed form of chromatin) located to both sides of centromeres

**Euchromatin:** a form of chromatin that is lightly packed

**Pleuropodia:** limb-derived glandular organs that transiently appear on the first abdominal segment in insect embryos

**Hox genes:** a group of genes that specify the body plan of an embryo along the head–tail axis of animals

**Maternal-lethal:** a recessive mutation that is viable in zygotes but that causes homozygous mutant mothers to produce inviable offspring

**Maternal-effect dominant embryonic arrest (*Medea*):** selfish genetic elements composed of a toxin and an antidote that are widespread in *T. castaneum* populations

infestation resulting in food adulteration and customer complaints that negatively impact brands and from increased costs resulting from returned products and required treatments.

## GENETICS

*Tribolium castaneum* is an excellent model organism for classical genetics, molecular genetics, population genetics, and epigenetics (49, 82). Its advantages as a model include easy rearing and handling, relatively short generation time, long adult lifespan, high fecundity, and high efficacy of genetic manipulation (142). Its global distribution and some unique genetic characteristics make it a common model for studying population genetics. Satellite DNAs, among the most intriguing and fascinating parts of the eukaryotic genome, are tandemly repeated sequences clustered within heterochromatin (19). In *T. castaneum*, satellite DNAs make up a substantial portion of the genome and represent major constituents of pericentromeric heterochromatin. Early studies of satellite DNA did not identify population-specific sequence features in individual satellite DNAs, but it was not clear if this was due to lack of population structure or because of the relatively limited numbers of cloned repeats (for a summary, see 49). Demuth & Wade (35) found genetic variation among global populations, including differentiation among some populations that resulted in hybrid breakdown, but this result was not correlated with geographic or genetic distance. They proposed that this variation is a consequence of nonadditive genetic effects and structured populations. The major satellite DNA TCAST1 is found partially dispersed within euchromatin (19). Such an organization, together with transcriptional activity, allows TCAST1 to modulate the activity of neighboring genes. Using a minor satellite DNA, TCAST2, Feliciello et al. (49) found population-specific satellite DNA profiles in *T. castaneum* and suggested the potential influence of satellite DNA dynamics on species evolution. Future research is needed to better understand population structure and potential speciation in *T. castaneum*, to link dispersal behaviors to population-level genetic variation, and to link genetic variation to phenotypic variation at the population level.

*Tribolium castaneum* has also been a popular model for studying developmental and evolutionary biology (18, 34, 85, 131). Like other coleopterans, *T. castaneum* develops pleuropodia, A1 appendages that have been conserved among most insect orders, but lacks larval appendages on the more posterior abdominal segments A2–A8 (84). It has been shown that the homeotic selector (Hox) gene *abdominal-A* (*abd-A*) serves an ancestral role in abdominal appendage development through the suppression of initiation of the appendage-promoting gene *Distal-less* (*Dll*), whereas another Hox gene, *Ultrabithorax* (*Ubx*), modulates abdominal appendage morphology in *T. castaneum* (84). In *Drosophila melanogaster* and many other insects, however, both *Ubx* and *abd-A* suppress appendage formation on most or all abdominal segments (80, 136). Differences have also been found in regulatory gene function in tracheal system development between *T. castaneum* and *D. melanogaster* (34). These findings highlight the importance of *T. castaneum* as a different model for studying functional variations of regulatory genes. To date, however, research in Hox genes has mainly focused on developmental and evolutionary biology. Further research is needed to examine possibilities for developing novel insect pest management strategies by targeting the Hox system in *T. castaneum* and other insect pest species.

One of the most fascinating phenomena found in *T. castaneum* is the combination of maternal-lethal and zygotic-rescue activities to gain a postzygotic survival advantage (15). Maternal-effect dominant embryonic arrest (*Medea*) involves two tightly linked loci, one encoding a lethal, maternally expressed toxin in all eggs and the other encoding a zygotic antidote that rescues only those progeny inheriting at least one *Medea* allele (14, 87). There has been interest in using *Medea* to suppress *T. castaneum* populations (133), but *Medea*'s patchy distribution in populations suggests

that released synthetic *Medea* may not spread uniformly, especially in *T. castaneum*, which has limited long-range dispersal (30). Nevertheless, such a *Medea*-based gene drive system may hold great promise for managing insect pest species in which its population distribution is localized and in which migration of wild types into the population is absent. It is also important to minimize the fitness cost of the drive and resistance to the toxin so that the drive is capable of spreading to fixation and maintaining itself in a population for years in the field.

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**RNA interference (RNAi):** small RNA-mediated downregulation of gene expression

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## GENOMICS AND FUNCTIONAL GENOMICS

*Tribolium castaneum* was the first coleopteran species whose genome was sequenced (115). The sequence (NCBI Tcas3.0) was based on a Sanger 7x draft assembly comprising 160 megabases and encoding approximately 16,500 genes. The *T. castaneum* genome is 33% bigger and encodes 6.5% more genes than that of *D. melanogaster* (115) and was further updated to provide more comprehensive genomic information two years later (75). Although *D. melanogaster* and *T. castaneum* share approximately 10,000–15,000 genes, *T. castaneum* is more representative of other insect species in many aspects of biology, particularly embryonic development (115). The *T. castaneum* genome has served as an important resource for various studies related to identification of new targets for pest management (79, 103, 146).

More recently, a new *T. castaneum* genome assembly (NCBI Tcas5.2) and an enhanced genome annotation with a new official gene set (OGS3) have become available (63). The authors of this work used new sequencing and mapping techniques to enhance the genomic resources, including a fivefold increase of scaffold length, improved precision of gene models, additional features such as untranslated regions (UTRs) and alternative splice variants to many gene models, and the addition of newly predicted genes. These improved genomic resources will facilitate much-needed research in functional genetics and comparative genomics. There is also a wealth of transcriptomic resources and studies for *T. castaneum*, including gene expression profiles at different developmental stages and in different tissues (38, 108, 109) and studies of stress responses (16, 56) and insecticide resistance (103) (see the sidebar titled *Tribolium castaneum* as an Animal Model for Biomedical Studies).

It has been noted that *T. castaneum* surpasses *Drosophila* in many aspects of genomics studies, especially those associated with embryonic development and RNA interference (RNAi)-mediated gene silencing (82, 135). Indeed, *T. castaneum* is an excellent insect model for RNAi-mediated gene silencing research due to its robust systemic RNAi responses through injection of double-stranded RNA (dsRNA) at all developmental stages (135). *Tribolium castaneum* has been used for high-throughput RNAi screening, including for G protein-coupled receptors (13), RNAi phenotypes (39), target genes for pest management (79, 138), embryonic and postembryonic development (123), wing and muscle development (86, 124), RNAi pathway genes (135), and genome-wide gene expression responses to RNAi or RNAi sequencing (RNAiSeq) (104). The wealth of

### TRIBOLIUM CASTANEUM AS AN ANIMAL MODEL FOR BIOMEDICAL STUDIES

Because insects are more ethically acceptable for biomedical research, the abundant genomic resources and genomic research tools have also made *T. castaneum* an increasingly used animal model for a variety of biomedical studies, including of neurodegenerative disorders (e.g., Parkinson's disease), the diuretic signaling pathway (vasopressin-like peptide and its receptor function), host-pathogen interactions (antagonistic interactions and coevolution), and pharmacology and toxicology (effects of psychoactive drugs) (3).

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**CRISPR/Cas9:**

a gene-editing technology for highly specific and rapid modification of DNA in a genome

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genomic resources and the readily available genomic research tools have made *T. castaneum* a popular insect model for various functional genomics studies, including studies of embryogenesis (112), development (121), the olfactory system and olfactory responses (37, 44), cuticle formation and pigmentation (100, 101), molting (20), chitin biosynthesis and metabolism (9, 102), immune responses (67), sex determination (126), metamorphosis (127), behavior (74), RNAi mechanisms (145), insecticide modes of action (89), and insecticide resistance (146).

These and many other studies using *T. castaneum* as a model for functional genomic studies have not only unveiled the functions of new genes and the new functions of old genes, but also facilitated functional genomic studies in many nonmodel insect species. For example, *T. castaneum* has been successfully used as a model to identify susceptible target genes for RNAi in agricultural insect pests such as *Diabrotica virgifera virgifera* and *Meligethes aeneus* (79). However, it is necessary to exercise caution when the susceptible target genes identified in *T. castaneum* are used to silence the orthologous genes in other insect species, as the efficiency of RNAi-mediated gene silencing is not only gene dependent but also insect species dependent. In addition, the vast majority of RNAi studies in *T. castaneum* have used injection as a dsRNA delivery method. Although injection is highly effective in silencing many target genes in *T. castaneum*, the RNAi efficiency could be different even for the same target gene when dsRNA is delivered by oral feeding because of different dynamics of dsRNA between injection and feeding (138). For insect pest management purposes, it would be necessary to deliver dsRNA by spray or feeding. However, research on dsRNA delivery using noninjection approaches is very limited in *T. castaneum*. A few studies have shown that RNAi-mediated gene silencing can also be effectively achieved by feeding *T. castaneum* larvae with dsRNA-incorporated flour discs (e.g., 29). Nevertheless, more research is needed to develop novel dsRNA delivery methods using *T. castaneum* as a model.

Recently, CRISPR/Cas9-mediated genome editing has also been established in *T. castaneum* (4, 54). Using the CRISPR/Cas9 system, researchers can now generate transgenic lines with site-specific insertions at their region of interest (45). This technology creates additional research opportunities to address fundamental and applied questions related to behavior and ecology and provides a platform for genetic intervention in pest management. Research in these areas can potentially lead to the development of new strategies for managing *T. castaneum* and other beetle pests.

## OLFACTION

Olfaction provides information to an organism about its environment and plays a critical role in guiding insect behavior. *Tribolium castaneum* has approximately 230 olfactory sensilla with approximately 600 odorant receptor neurons in their antennae (37, 40). In the *T. castaneum* olfaction system, 50 odorant binding proteins (OBPs), 20 chemosensory proteins (CSPs), 338 odorant receptors (ORs), 207 gustatory receptors, 23 ionotropic glutamate-like receptors, and 7 sensory neuron membrane proteins have been identified (37, 44). The *T. castaneum* genome has a larger number of OR genes compared to that of other evaluated insect species, but some of these genes may be nonfunctional or not expressed under all developmental or environmental conditions (44). OR gene expression was detected in both larval and adult head tissue (41 and 111 *TcOr* genes, respectively), but other genes were expressed only in adult bodies or in both adult bodies and heads. Although most *T. castaneum* CSPs and OBPs are transcribed in chemosensory tissues, others may be involved in other tasks and found in other tissues (38). In *T. castaneum* larvae, the functional and morphological classes of sensory genes were found to have diverged from homologous classes in *D. melanogaster* sensilla (77). These results led to the proposal of an evolutionarily flexible gene network scenario, where sensory genes vary in roles, regulation, and



## ABUNDANT OLFACTORY GENES MAY ENABLE *TRIBOLIUM CASTANEUM* TO EXPLOIT DIVERSE LANDSCAPES

Some have found it surprising that so many olfactory genes are needed by *T. castaneum*, given that, in some current ecological niches, they can be literally surrounded by food; however, this pattern may not be a common feature of their environments. *Tribolium castaneum* can often be found exploiting much more diverse and complex landscapes, and the role of chemosensory processes in resource finding is likely to be an important, although not well understood, part of this species' success.

temporal expression. High diversity in olfactory genes might contribute to this species' ability to find and infest a wide range of resources and to find mates and avoid risks in spatially and temporally complex landscapes (see the sidebar titled Abundant Olfactory Genes May Enable *Tribolium castaneum* to Exploit Diverse Landscapes).

Olfactory responses can depend on genetic, developmental, and learned influences, and research into the neurobiology of *T. castaneum* has provided some interesting insights. Kenyon cells in the *T. castaneum* brain are involved with learning and memory, and cell proliferation is primarily genetically controlled during early stages of development, while in later stages, proliferation is influenced by the odor environment (137). Isolated beetles, not exposed to pheromone or other conspecific cues, had a shorter new proliferation phase than beetles exposed to pheromone or a food odor (*cis*-3-hexenol) (137). Knocking down expression of an olfactory gene, which eliminated electroantennography (EAG) response to pheromone and lowered response to *cis*-3-hexenol, shortened the duration of the prolonged Kenyon cell proliferation phase observed in the presence of conspecifics. This linkage between sensory input and adult neurogenesis and the large number of OR genes might give beetles the flexibility to learn new odorant memories and contribute to behavioral variation among beetle populations. This ability may contribute to the ecological success of *T. castaneum* but also negatively impact human ability to monitor or disrupt resource finding across pest populations in different types of food facilities.

Behavioral responses of adults to kairomone and pheromone odors have been investigated, but response levels are often relatively low and strongly influenced by endogenous and exogenous factors. The functional role of aggregation pheromone and other volatile cues associated with food and infested foods is not well understood for this species, despite extensive research. Dispersal by walking is common in *T. castaneum*, and most research evaluating behavior has used walking adults. Several experimental approaches have been developed to measure behavioral responses to stimuli, but the level of response can vary with the approach, as well as with strain (53). Differences in response to volatile cues among populations have a genotype component but also have a high environmental coefficient of variation (53). *Tribolium castaneum* adults exhibited low attraction to kairomone and pheromone cues in traps under still air conditions but strong positive response to pheromone and the combination of pheromone and kairomone with air flow (23). Being raised on low-nutrition diets increased adult response to pheromone, as measured by captures in pheromone-baited traps, with unmated adults responding more strongly than mated individuals (47). *Tribolium castaneum* pheromone is a blend of four stereoisomers, and attraction in a wind tunnel was influenced by the number and ratio of the stereoisomers (88). The ability to manipulate gene expression using RNAi- or transposon-mediated transformation is a powerful tool that could be used to determine both the mechanistic roles of olfaction in finding and exploiting food resources and the adaptive value of these traits in exploiting spatially and temporally patchy resources. While research taking this approach has been limited to date, one study has shown that injecting pupae with *TiOr1* dsRNA eliminated adult response to aggregation pheromone (44).

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### Pheromone:

a chemical substance secreted by an organism affecting the behavior or physiology of other individuals of the same species

### Electroantennography

(EAG): a technique for measuring the electrical activity of an insect antenna

### Kairomone:

a chemical substance emitted by an organism and detected by an individual of a different species that thereby gains benefits

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**Meta-analysis:**

examination of data from several independent studies of the same research question

**Patch:** an area of habitat, often the smallest ecologically distinct area, that is different from the surrounding environment

**Anemotaxis:**

movement of an organism in response to air current

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*Tribolium castaneum* olfactory responses have been exploited in pest management programs, with considerable research conducted on the implementation and interpretation of *T. castaneum* monitoring programs and a variety of attractants and traps available commercially. In bulk grains, walking *T. castaneum* can be detected with perforated probe and pitfall traps, and captures in traps correlate well with presence in grain samples and can be linked with action thresholds (11). However, the most commonly used monitoring devices for this species are floor pitfall-type traps baited with pheromone and kairomone that capture walking adults, and these traps have been used with success in structures where food materials are processed and stored. Results of trapping programs have been used to illustrate the spatiotemporal distribution of insects in different types of storage facilities (27, 28), broad patterns in treatment efficacy using meta-analysis techniques (24), population rebound after treatment, the influence of season on colonization from outside sources, and the impact of sanitation in making the landscape less favorable for colonization (28). Understanding the functional genomics of *T. castaneum* response to chemical cues also creates the potential to identify specific genes, which may be targeted for disruption to reduce the damage caused by this pest. Key to this success will be the development of delivery systems, which, given the nature of the postharvest environment, will be different from the approaches taken in field crops.

## LANDSCAPE BEHAVIOR AND ECOLOGY

*Tribolium castaneum* is a useful species for the evaluation of movement and the exploitation of spatially and temporally patchy landscapes. Experimental microlandscapes using patches of flour have been widely used, from simple choice tests to complex neutral landscape models with different levels of abundance and fragmentation. *Tribolium castaneum* can detect and respond to landscape structure, with adults moving more slowly and tortuously, and remaining longer in both the overall landscape and individual patches, in fine-grained landscapes than in coarse-grained landscapes (117). The proportion of beetles successfully locating a patch decreased as a function of distance, and beetles exhibited positive anemotaxis whether flour was present in the patch or not (118). In these studies, physical encounters with flour patches may be more significant in manipulating movement than longer-range responses to volatile cues from the patches.

*Tribolium castaneum* patch exploitation behavior has been evaluated. Beetles recognize the amount of resource in a patch and adjust oviposition so that they lay the predicted optimal egg number for a given resource amount (26). Physical characteristics of the patch also influenced the tendency to enter or leave, with leaving rates being lower in thicker patches or thinner patches that are covered (118). Under ideal free distribution (IFD), groups of organisms distribute themselves between patches in proportion to the suitability of each patch. In *T. castaneum*, groups of only females, which lack aggregation pheromone, demonstrated an IFD, but groups with pheromone (mixed-sex groups or female groups plus pheromone) undermatched the IFD in low-food habitats (61). Differences in patch quality can also influence oviposition decisions. Females often laid more eggs in foods that supported successful progeny development, but oviposition decisions were not always correct (52). Even within patches, selective feeding can impact patch quality: Stable isotope analysis was used to show that larvae in patches with different ratios of corn and wheat fed disproportionately to the ratio, suggesting that larvae can feed selectively on preferred flour particles (51). Gene expression manipulation effects on how beetles exploit landscapes and analysis of gene expression patterns under different landscape environments appear to be promising areas of research to understand how beetles exploit landscapes and how this process might be manipulated in pest management. Wild-type strains of *D. melanogaster* with natural allelic variants of the rover and sitter *forager* gene adopted different movement patterns under certain landscape patterns when evaluated on experimental landscapes with varying distribution and number of cells containing



food (42). This highlights how genotype expression can be influenced by environmental factors such as landscape pattern.

Successful colonization of a resource patch depends on the propagule pressure resulting from the number of individuals per introduction and number of introductions. In a study using microlandscape experiments and demographic simulations, the probability of establishment was greatest with multiple smaller introductions than with one or two larger introductions (81). Experimental establishment probabilities equaled or exceeded the demographic model simulations, and population growth rates also exceeded predictions, perhaps due to rapid genetic adaptation. Rapid evolution during range expansion can occur (143), and constraining evolution can reduce establishment and spread (132). In addition to propagule pressure, prior adaptation to the environment can also play a role in establishment success (139). Microlandscape experiments have also been used to assess conservation strategies for small populations, with both genetic and demographic rescue resulting from immigration acting additively to reduce extinction (66).

The ways in which *T. castaneum* interacts with spatially and temporally patchy landscapes also influence other aspects of this species' biology. Both male and female *T. castaneum* mate multiple times and have been used to investigate the fitness benefits of polyandry and mechanisms of cryptic female choice (46, 48, 105, 106). Genetic bottlenecks led to increased female promiscuity compared to noninbred lines, suggesting that mating patterns can rapidly change in response to shifts in costs and benefits (95). Death-feigning behavior is a defense mechanism against predators, and in *T. castaneum*, it has a genetic component and can improve survival of encounters with predators (97). Strains that had been selected for greater dispersal activity also had a lower intensity of death-feigning behavior (93) and a greater risk of predation (91). Selection for increased mobility versus increased death-feigning behavior was also correlated with changes in morphology, with longer dispersal lines having longer legs (92).

The process of dispersal from resource patches is important in predicting population spread and has been investigated using *T. castaneum* as a model. Dispersal distance has a genetic component, and strains have been selected for high and low dispersal ability (91). The tendency to disperse in *T. castaneum* is a polygenic trait that has epistatic interactions. Analysis of lines selected for high and low dispersal indicated that epistatic variation may be converted to additive variance and fixed in the selected lines, suggesting that epistatic variation might be an adaptive reserve that can be used in small populations (120). Density also influences dispersal, with greater emigration occurring under high-density than under low-density conditions (43, 59). Juvenile environment also influences adult dispersal tendency and distance: Adults exposed to a poor environment disperse farther if they were also exposed to a poor environment as a juvenile or if they are exposed to a group of other adults in which more than one-third were exposed to a poor environment as juveniles (43). Interspecific competition can also impact dispersal (83). The mechanisms by which adults make these assessments about dispersal are not known but likely involve responses to chemical signaling.

Understanding pest distribution and movement patterns within landscapes can help guide pest monitoring programs and the selection and application of management tactics. Adults walk at an average speed of 14 cm/min but are active only 26% of the time (25). However, in food facilities, adults are able to move among floors in a flour mill (125), broad spatial distribution patterns can change over time, and food facilities can be rapidly recolonized, suggesting a high level of mobility (27, 28). Dispersal outside of food facilities is less well understood, but adults can fly, and dispersing beetles have been captured up to 300 m and 1 km from likely sources (57, 116). Given this level of mobility, the patchy resource landscape at food facilities, and the placement of traps outside of resource patches, it is challenging to interpret monitoring programs, and this is where a better understanding of how beetles exploit landscape structure has

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**Insect growth**

**regulator:** a chemical insecticide that functionally mimics hormones to disrupt the insect life cycle

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been important. Trap catch is influenced by many aspects of biology that need to be understood and considered in interpreting captures in traps and making pest management decisions (<https://storedproductinsects.com/biology/interpretation-of-trap-catch/>), and refinements in the implementation and interpretation of monitoring programs will require greater understanding of these factors. Conclusions about the relationship between adult captures in traps and insect density in a facility can be difficult to make, and this relationship has been a research focus using *T. castaneum* as a model. There has often been a weak relationship between capture in traps and infestation levels in equipment (62), as traps primarily capture individuals dispersing between resource patches, but in model systems, these relationships between abundance and capture have been more clear (22). Application of insecticides can also impact the relationship between insect density and adults captured in traps. Cyfluthrin insecticide applications reduced insect dispersal and capture in traps but did not significantly impact the populations in hidden food patches (134).

## INSECTICIDE EFFICACY AND RESISTANCE

Insecticides are commonly used in pest management programs, but there is increasing emphasis on making applications more targeted and using reduced-risk materials. *Tribolium castaneum* has been widely used in research evaluating the efficacy of traditional and novel insecticides not only because of its importance as a pest, but also because it is often a good indicator species, given that it tends to have lower susceptibility to many insecticides. Insecticides can be used to treat food material directly, typically only for bulk stored raw grains, or to treat surfaces with which insects are likely to come into contact during dispersal, and can be applied as a gas (fumigant), aerosol, or spray or incorporated into materials such as packaging or netting. There has been a lot of focus on the use of reduced-risk insecticides. With pyrethrins and pyrethroids, efficacy varies with active ingredient, application method, and developmental stage (73). With the bacterial insecticide spinosad, *T. castaneum* was far less susceptible than were other stored product beetle species (64). Insect growth regulators, such as S-methoprene, are effective in controlling *T. castaneum* immature development and progeny production capacity (144). Inert materials, such as diatomaceous earths and zeolites, can be effective, but *T. castaneum* is considered to be less susceptible to these materials than are other key stored product insect species (78). *Tribolium castaneum* is probably the most thoroughly examined species for its susceptibility to the fumigant phosphine, due in part to the occurrence of strongly phosphine-resistant populations in several parts of the world (99). In populations that are not resistant, phosphine is effective as long as good fumigation practices are applied (5), with eggs being the most tolerant life stage (114).

In the case of most pyrethrins and pyrethroids, exposure to insecticide causes rapid knockdown, from which insects either die or recover. The presence of flour during or after treatment promotes insect recovery, highlighting the importance of sanitation in controlling this species (10). The factors that contribute to recovery from knockdown, including how exposure to flour influences this response, are not well understood. The status of *T. castaneum* as a genomics model has enabled its use to evaluate mechanisms of detoxification (71) and resistance (103, 146).

Resistance to insecticides has evolved frequently in *T. castaneum* (32). More recently, multiple resistance to organophosphates and pyrethroids in *T. castaneum* has been reported, which complicates further the use of traditional insecticides for the control of this species (71). *Tribolium castaneum* has been a model species in studying phosphine resistance (68). It was the first species in which it was confirmed that phosphine resistance is related to the dimerization interface of dihydrolipoamide dehydrogenase (122), and it has been shown that resistance involves two genes, *rph1* and *rph2*, with the latter being responsible for the high level of resistance (69). Cytochrome P450 CYP346 family genes are also associated with resistance; these genes are overexpressed

in resistant populations and upregulated following exposure to phosphine (141). Populations of *T. castaneum* have been characterized as strongly resistant to phosphine (32), but survival following phosphine fumigation can result from both resistance and poor fumigation practices (5). Resistant populations of *T. castaneum* have been used as models to evaluate certain behavioral, longevity, and fecundity fitness costs, which may further complicate resistance monitoring and management (68). Phosphine-resistant *T. castaneum* strains have been reported to have a lower respiration rate (110), to walk more slowly, and to locate food resources less frequently than do beetles from a susceptible strain (90), suggesting that phosphine-resistant strains may have reduced dispersal ability in broader landscapes. Colonization behavior also impacts insecticide resistance management: Phosphine resistance increased slowly in populations under low immigration and in the absence of selection, as expected, but at higher immigration rates, the increase in frequency of the resistance allele was lower than predicted (113). This is likely due to high levels of polyandry with immigrating resistant females, resulting in increased heterozygosity in progeny. Evaluation of behavior can also help in assessing resistance levels in populations. Reduction in normal movement following exposure to phosphine can potentially be used as a diagnostic tool, since time to immobilization was inversely related to time to recovery (12).

A strong foundation of information related to insecticide efficacy and genetics and genomics tools makes this species a good model to further assess modes of action of insecticides and mechanisms for recovery, low susceptibility, and resistance, which could improve the effectiveness of treatments. *Tribolium castaneum* is also a useful model for the identification of new pest management gene targets and the development of new targeted insecticidal delivery methods such as using RNAi (109). Application of these approaches in food facilities such as grain bins or flour mills presents some unique challenges, and it is unlikely that genetic manipulation of crops will prove to be the best method to deliver new biologically based insecticides. Use of treated attractive baits is likely to be the more fruitful approach to targeting genes. This is consistent with recent trends in pest management that have focused on reduced-risk materials applied in more targeted ways. This type of approach requires an integrated understanding of insecticide modes of action, along with pest insect behavior and ecology within food facility landscapes. For example, incorporation of insecticide into materials such as packaging materials or nets is an approach that targets insecticide treatments at critical points to reduce dispersal and colonization (72, 98). Manipulation of behavior and exploitation of behavioral patterns through approaches such as lure-and-kill and push-pull, with a focus on prevention of infestations, are particularly promising.

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**Economic threshold:**  
a pest density at which  
a control treatment  
will provide an  
economic return

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## DECISION MAKING IN PEST MANAGEMENT

Several modeling approaches have been developed to help guide *T. castaneum* pest management programs, although in practice, treatments in food facilities are most often calendar based. Computer simulation models have been developed as decision aids for management in stored raw wheat (58). A decision support system was developed for predicting when to fumigate in bagged rice stacks (65). Equations describing the relationship among *T. castaneum* density, duration of infestation, and weight loss or damage of cocoa beans have been developed to forecast whether damage will exceed economic thresholds (1). Potential patterns of global distribution of *T. castaneum* in cocoa cultivation areas have been predicted and estimates of damage and economic losses have been developed for those countries (70). Relatively few studies have focused on optimizing pest management decisions (58), but this is essential to maximizing efficacy. Considerably less decision support information is available for the more complex environments found in food processing facilities and warehouses, where the challenges of landscape utilization and monitoring discussed above are prevalent. Risk threshold levels for *T. castaneum* captures in traps (28) and a model of the

population impacts of methyl bromide fumigations (50) have been developed for flour mills. Ultimately, it will be necessary to integrate into these decision models the roles of immigration and emigration, temporal and spatial landscape structure impacts on movement and populations, and the specific targeted effects of treatments to make the models more functional. Advances in the more fundamental research on behavior and ecology provide valuable information on which to build this support system.

### SUMMARY POINTS

1. *Tribolium castaneum* is one of the most ubiquitous and most studied stored product insects, with a nearly worldwide distribution, a broad host range, and economic importance as a pest.
2. *Tribolium castaneum* is a widely used model for classical genetics, molecular genetics, population genetics, and epigenetics and an emerging model for high-throughput RNAi screening and functional genomics studies.
3. Using *T. castaneum* as a model to assess olfaction and the influence of landscape structure on behavior and ecology using microlandscapes have been exciting areas of research, but linkages among these different areas have been limited, and manipulation of gene expression to determine the functional value of the many olfactory genes needs to be undertaken.
4. *Tribolium castaneum* as a model for applied research extends beyond postharvest management into research on aging, biomedicine, the environment, livestock breeding, and pest management.

### FUTURE ISSUES

1. Available genomic knowledge, resources, and tools such as gene silencing need to be better integrated into behavioral and ecological research using *T. castaneum* as a model.
2. Functional studies of the *T. castaneum* sensory system are needed to help determine how behavioral responses increase fitness and how disruption of these responses can be used for better pest management.
3. Research on mechanisms for *T. castaneum* low susceptibility to specific insecticides, recovery from knockdown, and insecticide detoxification and resistance is needed.
4. Target genes for developing novel eco-friendly pest management strategies need to be identified, and delivery methods need to be developed and tested to determine their efficacy for pest management under real-world conditions.
5. Improved decision-making methods informed by an understanding of behavior and ecology need to be developed for pest management.

### DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and

does not imply recommendation or endorsement by the US Department of Agriculture (USDA), the University of Thessaly, or Kansas State University. The USDA and Kansas State University are equal opportunity providers and employers.

## LITERATURE CITED

1. Abdullahi G, Muhamad R, Dzolkhiffi O, Sinniah UR. 2018. Damage potential of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) on cocoa beans: effect of initial adult population density and post infestation storage time. *J. Stored Prod. Res.* 75:1–9
2. Abdullahi G, Muhamad R, Sule H. 2019. Biology, host range and management of red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): a review. *Taraba J. Agric. Res.* 7:48–64
3. Adamski Z, Bufo SA, Chowanski S, Falabella P, Lubawy J, et al. 2019. Beetles as model organisms in physiological, biomedical and environmental studies: a review. *Front. Physiol.* 10:319
4. **Adrianos S, Lorenzen M, Oppert B. 2018. Metabolic pathway interruption: CRISPR/Cas9-mediated knockout of tryptophan 2,3-dioxygenase in *Tribolium castaneum*. *J. Insect Physiol.* 107:104–9**
5. Agrafioti P, Sotiropoulos V, Kaloudis S, Bantas S, Bochtis D, Athanassiou CG. 2020. Real time monitoring of phosphine and insect mortality in different storage facilities. *J. Stored Prod. Res.* 89:101726
6. Aitken AD. 1975. *Insect travelers. Volume I. Coleoptera. A survey of the beetles recorded from imported cargoes by the insect inspectorate of the Ministry of Agriculture, Fisheries and Food with a special analysis for the years 1957 to 1969.* Tech. Bull. 31, HMSO, London
7. Alabi T, Michaud JP, Arnaud L, Haubrugge E. 2008. A comparative study of cannibalism and predation in seven species of flour beetle. *Ecol. Entomol.* 33:716–26
8. Altincicek B, Knorr E, Vilcinskas A. 2008. Beetle immunity: identification of immune-inducible genes from the model insect *Tribolium castaneum*. *Dev. Comp. Immun.* 32:585–95
9. Arakane Y, Muthukrishnan S, Kramer KJ, Specht CA, Tomoyasu Y, et al. 2005. The *Tribolium* chitin synthase genes *TcCHS1* and *TcCHS2* are specialized for synthesis of epidermal cuticle and midgut peritrophic matrix. *Insect Mol. Biol.* 14:453–463
10. Arthur FH. 2013. Dosage rate, temperature, and food source provisioning affect susceptibility of *Tribolium castaneum* and *Tribolium confusum* to chlorfenapyr. *J. Pest Sci.* 86:507–13
11. Athanassiou CG, Buchelos CT. 2001. Detection of stored-wheat beetle species and estimation of population density using unbaited probe traps and grain trier samples. *Entomol. Exp. Appl.* 98:67–78
12. Athanassiou CG, Kavallieratos NG, Brabec DL, Oppert B, Guedes RNC, Campbell JF. 2019. From immobilization to recovery: towards the development of a rapid diagnostic indicator for phosphine resistance. *J. Stored Prod. Res.* 80:28–33
13. Bai H, Zhu F, Shah K, Palli SR. 2011. Large-scale RNAi screen of G protein-coupled receptors involved in larval growth, molting and metamorphosis in the red flour beetle. *BMC Genom.* 12:388
14. Beeman RW, Arakane Y, Phillips TW, Muthukrishnan S. 2010. Implications of the *Tribolium* genome project for pest biology. In *Proceedings of the 10th International Working Conference on Stored Product Protection*, ed. MO Carvalho, PG Fields, CS Adler, FH Arthur, CG Athanassiou, et al., pp. 63–71. Berlin: Julius Kühn Inst.
15. **Beeman RW, Friesen KS, Denell RE. 1992. Maternal-effect selfish genes in flour beetles. *Science* 256:89–92**
16. Behrens S, Peuß R, Milutinović B, Eggert H, Esser D, et al. 2014. Infection routes matter in population-specific responses of the red flour beetle to the entomopathogen *Bacillus thuringiensis*. *BMC Genom.* 15:445
17. Bell AE. 1982. The *Tribolium* model in animal breeding research. In *Proceedings of the Second World Congress on Genetics and Applications to Livestock Production, Madrid, Spain*, Vol. 5, pp. 26–42. N.p., WC-GALP
18. Benton MA, Pavlopoulos A. 2014. *Tribolium* embryo morphogenesis: May the force be with you. *Bioarchitecture* 4:16–21
19. Brajković J, Pezer Ž, Bruvo-Madarić B, Sermek A, Feliciello I, Ugarković Đ. 2018. Dispersion profiles and gene associations of repetitive DNAs in the euchromatin of the beetle *Tribolium castaneum*. *G3* 8:875–86

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4. Describes the application of the CRISPR/Cas9 system to create a more defined mutation in *T. castaneum*.

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15. Illustrates the *Medea* genes in *T. castaneum* in relation to progeny production capacity.

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20. Broehan G, Arakane Y, Beeman RW, Kramer KJ, Muthukrishnan S, et al. 2010. Chymotrypsin-like peptidases from *Tribolium castaneum*: a role in molting revealed by RNA interference. *Insect Biochem. Mol. Biol.* 40:274–83
21. Brown SJ, Shippy TD, Miller S, Bolognesi R, Beeman RW, et al. 2009. The red flour beetle, *Tribolium castaneum* (Coleoptera): a model for studies of development and pest biology. *Cold Spring Harb. Protoc.* 8:pdb.emo126
22. Buckman KA, Campbell JF. 2013. How varying pest and trap densities affect *Tribolium castaneum* capture in pheromone traps. *Entomol. Exp. App.* 146:404–12
23. Campbell JF. 2012. Attraction of walking *Tribolium castaneum* adults to traps. *J. Stored Prod. Res.* 51:11–22
24. Campbell JF, Buckman KA, Fields PG, Subramanyam B. 2015. Evaluation of structural treatment efficacy against *Tribolium castaneum* and *Tribolium confusum* using meta-analysis of multiple studies conducted in food facilities. *J. Econ. Entomol.* 108:2125–40
25. Campbell JF, Hagstrum DW. 2002. Patch exploitation by *Tribolium castaneum*: movement patterns, distribution and oviposition. *J. Stored Prod. Res.* 38:55–68
26. Campbell JF, Runnion C. 2003. Patch exploitation by female red flour beetles, *Tribolium castaneum*. *J. Insect Sci.* 3:20
27. Campbell JF, Toews MD, Arthur FH, Arbogast RT. 2010. Long-term monitoring of *Tribolium castaneum* populations in two flour mills: seasonal patterns and impact of fumigation. *J. Econ. Entomol.* 103:991–1001
28. Campbell JF, Toews MD, Arthur FH, Arbogast RT. 2010. Long-term monitoring of *Tribolium castaneum* populations in two flour mills: rebound after fumigation. *J. Econ. Entomol.* 103:1002–11
29. Cao M, Gatehouse JA, Fitches EC. 2018. A systematic study of RNAi effects and dsRNA stability in *Tribolium castaneum* and *Acyrtosiphon pisum*, following injection and ingestion of analogous dsRNAs. *Int. J. Mol. Sci.* 19:1079
30. Cash SA, Lorenzen MD, Gould F. 2019. The distribution and spread of naturally occurring *Medea* selfish genetic elements in the United States. *Ecol. Evol.* 9:14407–16
31. Chandrasekar R, Palli SR. 2011. Reproductive biology of *Tribolium castaneum*. In *Entomology, Ecology & Biodiversity*, ed. BK Tyagi, V Veer, pp. 197–215. Jodhpur, India: Sci. Publ.
32. Collins PJ, Schlipalius DI. 2018. Insecticide resistance. In *Recent Advances in Stored Product Protection*, ed. CG Athanassiou, FH Arthur, pp. 169–82. Berlin: Springer
33. Costantino RF, Desharnais RA. 2012. *Population Dynamics and the Tribolium Model: Genetics and Demography*. Berlin: Springer
34. de Miguel C, Linsler F, Casanova J, Franch-Marro X. 2016. Genetic basis for the evolution of organ morphogenesis: the case of spalt and cut in the development of insect trachea. *Development* 143:3615–22
35. Demuth JP, Wade MJ. 2007. Population differentiation in the beetle *Tribolium castaneum*. I. Genetic architecture. *Evolution* 61:494–509
36. Denell R. 2008. Establishment of *Tribolium* as a genetic model system and its early contributions to evo-devo. *Genetics* 180:1779–86
37. Dippel S, Kollmann M, Oberhofer G, Montino A, Knoll C, et al. 2016. Morphological and transcriptomic analysis of a beetle chemosensory system reveals a gnathal olfactory center. *BMC Biol.* 14:90
38. Dippel S, Oberhofer G, Kahnt J, Gerischer L, Opitz L, et al. 2014. Tissue-specific transcriptomics, chromosomal localization, and phylogeny of chemosensory and odorant binding proteins from the red flour beetle *Tribolium castaneum* reveal subgroup specificities for olfaction or more general functions. *BMC Genom.* 15:1141
39. Dönitz J, Schmitt-Engel C, Grossmann D, Gerischer L, Tech M. 2015. iBeetle-Base: a database for RNAi phenotypes in the red flour beetle *Tribolium castaneum*. *Nucleic Acids Res.* 43:D720–25
40. Dreyer D, Vitt H, Dippel S, Goetz B, Jundi B, et al. 2010. 3D standard brain of the red flour beetle *Tribolium castaneum*: a tool to study metamorphic development and adult plasticity. *Front. Syst. Neurosci.* 4:3
41. Dyte CE, Blackman DG. 1970. The spread of insecticide resistance in *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *J. Stored Prod. Res.* 6:255–61
42. Edelsparre AH, Fitzpatrick MJ, Rodriguez MB, Greenspan RJ. 2020. Tracking dispersal across a patchy landscape reveals a dynamic interaction between genotype and habitat structure. *Oikos* 130:79–94



43. Endriss SB, Vahsen ML, Bitume EV, Monroe JG, Turner KG, et al. 2019. The importance of growing up: Juvenile environment influences dispersal of individuals and their neighbours. *Ecol. Lett.* 22:45–55
44. Engsontia P, Sanderson AP, Cobb M, Walden KKO, Robertson HM, Brown S. 2008. The red flour beetle's large nose: an expanded odorant receptor gene family in *Tribolium castaneum*. *Insect Biochem. Mol. Biol.* 38:387–97
45. Farnworth MS, Eckermann KN, Ahmed HMM, Mühlen DS, He B, Bucher G. 2020. The red flour beetle as model for comparative neural development: genome editing to mark neural cells in *Tribolium* brain development. *Methods Mol. Biol.* 2047:191–217
46. Fedina TY. 2007. Cryptic female choice during spermatophore transfer in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. Insect Physiol.* 53:93–98
47. Fedina TY, Lewis SM. 2008. Effect of *Tribolium castaneum* (Coleoptera: Tenebrionidae) nutritional environment, sex and mating status on response to commercial pheromone traps. *J. Econ. Entomol.* 100:1924–27
48. Fedina TY, Lewis SM. 2008. An integrative view of sexual selection in *Tribolium* flour beetles. *Biol. Rev.* 83:151–71
49. Feliciello I, Akrap I, Brajković J, Zlatar I, Ugarković Đ. 2014. Satellite DNA as a driver of population divergence in the red flour beetle *Tribolium castaneum*. *Genome Biol. Evol.* 7:228–39
50. Flinn PW, Campbell JF, Throne JE, Subramanyam B. 2010. Simulation model of the red flour beetle in flour mills. In *Proceedings of the 10th International Working Conference on Stored Product Protection*, ed. MO Carvalho, PG Fields, CS Adler, FH Arthur, CG Athanassiou, et al., pp. 953–55. Berlin: Julius Kühn Inst.
51. Focken U. 2007. Effect of different ratios of wheat to corn flour in the diet on the development and isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of the red flour beetle *Tribolium castaneum*. *Isot. Environ. Health Stud.* 43:143–54
52. Gerken AR, Campbell JF. 2020. Oviposition and development of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) on different types of flour. *Agronomy* 10:1593
53. Gerken AR, Scully ED, Campbell JF. 2018. Red flour beetle (Coleoptera: Tenebrionidae) response to volatile cues varies with strain and behavioral assay. *Environ. Entomol.* 47:1252–65
54. Gilles AF, Schinko JB, Averof M. 2015. Efficient CRISPR-mediated gene targeting and transgene replacement in the beetle *Tribolium castaneum*. *Development* 142:2832–39
55. Good NE. 1936. *Flour beetles of the genus Tribolium*. Tech. Bull. 498, U. S. Dep. Agric., Washington, DC
56. Guo SH, Yu L, Liu YM, Wang F-F, Chen Y-C, et al. 2019. Digital gene expression profiling in larvae of *Tribolium castaneum* at different periods post UV-B exposure. *Ecotoxicol. Environ. Saf.* 174:514–23
57. Gurdasani K, Rafter MA, Daglish GJ, Walter GH. 2018. Characterising the variables associated with *Tribolium castaneum* adults that initiate flight in laboratory tests: generating predictions for the field. *J. Stored Prod. Res.* 79:123–31
58. Hagstrum DW, Flinn PW. 1990. Simulations comparing insect species differences in response to wheat storage conditions and management practices. *J. Econ. Entomol.* 83:2469–75
59. Hagstrum DW, Gilbert EE. 1976. Emigration rate and age structure dynamics of *Tribolium castaneum* populations during growth phase of a colonizing episode. *Environ. Entomol.* 5:445–48
60. Hagstrum DW, Subramanyam B. 2009. *Stored-Product Insect Resource*. St. Paul, MN: AACC Int.
61. Halliday WD, Blouin-Demers G. 2016. Male aggregation pheromones inhibit ideal free habitat selection in red flour beetles (*Tribolium castaneum*). *J. Insect Behav.* 29:355–67
62. Hawkin KJ, Stanbridge DM, Fields PG. 2011. Sampling *Tribolium confusum* and *Tribolium castaneum* in mill and laboratory settings: differences between strains and species. *Can. Entomol.* 143:504–17
63. Herndon N, Shelton J, Gerischer L, Ioannidis P, Ninova M, et al. 2020. Enhanced genome assembly and a new official gene set for *Tribolium castaneum*. *BMC Genom.* 21:47
64. Hertlein MB, Thompson GD, Subramanyam B, Athanassiou CG. 2011. Spinosad: a new natural product for stored grain protection. *J. Stored Prod. Res.* 47:131–46
65. Hodges RJ, Smith M, Madden A, Russell D, Gudrups I, Halid H. 1997. Development of a decision support system for the fumigation of milled-rice bag-stacks in the tropics. In *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products*, ed. EJ Donahaye, S Navarro, A Varnava, pp. 425–34. Nicosia, Cyprus: Printco Ltd.

68. Examines fitness cost of populations of *T. castaneum* that are resistant to phosphine.

81. Application of microlandscape experiments and demographic simulations to evaluate how colonization success is impacted by patterns of introductions.

66. Hufbauer RA, Szűcs M, Kasyon E, Youngberg C, Koontz MJ, et al. 2015. Three types of rescue can avert extinction in a changing environment. *PNAS* 112:10557–62
67. Jacobs CG, Spaik HP, van der Zee M. 2014. The extraembryonic serosa is a frontier epithelium providing the insect egg with a full-range innate immune response. *eLife* 3:e04111
68. Jagadeesan R, Collins PJ, Daglish GJ, Ebert PR, Schlipalius DI. 2012. Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): inheritance, gene interactions and fitness costs. *PLOS ONE* 7:e31582
69. Jagadeesan R, Fotheringham A, Ebert PR, Schlipalius DI. 2013. Rapid genome wide mapping of phosphine resistance loci by a simple regional averaging analysis in the red flour beetle, *Tribolium castaneum*. *BMC Genom.* 14:650
70. Jung J-M, Byeon D-H, Kim S-H, Sunghoon-Jung, Lee W-H. 2020. Estimating economic damage to cocoa bean production with changes in the spatial distribution of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in response to climate change. *J. Stored Prod. Res.* 89:101681
71. Kalsi M, Palli SR. 2017. Cap n collar transcription factor regulates multiple genes coding for proteins involved in insecticide detoxification in the red flour beetle, *Tribolium castaneum*. *Insect Biochem. Mol. Biol.* 90:43–52
72. Kavallieratos NG, Athanassiou CG, Arthur FH. 2017. Effectiveness of insecticide-incorporated bags to control stored-product beetles. *J. Stored Prod. Res.* 70:18–24
73. Kharel K, Arthur FA, Zhu KY, Campbell JF, Subramanyam B. 2014. Evaluation of synergized pyrethrin aerosol for control of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* 107:462–68
74. Kim HG, Margolies D, Park Y. 2015. The roles of thermal transient receptor potential channels in thermotactic behavior and in thermal acclimation in the red flour beetle, *Tribolium castaneum*. *J. Insect Physiol.* 76:47–55
75. Kim HS, Murphy T, Xia J, Caragea D, Park Y, et al. 2010. BeetleBase in 2010: revisions to provide comprehensive genomic information for *Tribolium castaneum*. *Nucleic Acids Res.* 38:D437–42
76. King CE, Dawson PS. 1972. Population biology and the *Tribolium* model. *Evol. Biol.* 5:133–227
77. Klann M, Schacht MI, Benton MA, Stollewerk A. 2021. Functional analysis of sense organ specification in the *Tribolium castaneum* larva reveals divergent mechanisms in insects. *BMC Biol.* 19:22
78. Kljajic P, Andric G, Adamovic M, Bodroza-Solarov M, Markovic M, et al. 2010. Laboratory assessment of insecticidal effectiveness of natural zeolite and diatomaceous earth formulations against three stored-product beetle pests. *J. Stored Prod. Res.* 46:1–6
79. Knorr E, Fishilevich E, Tenbusch L, Frey MLF, Rangasamy M, et al. 2018. Gene silencing in *Tribolium castaneum* as a tool for the targeted identification of candidate RNAi targets in crop pests. *Sci Rep.* 8:2061
80. Konopova B, Akam M. 2014. The *Hox* genes *Ultrabithorax* and *abdominal-A* specify three different types of abdominal appendage in the springtail *Orchesella cincta* (Collembola). *EvoDevo* 5:2
81. Koontz MJ, Oldfather MF, Melbourne BA, Hufbauer RA. 2018. Parsing propagule pressure: Number, not size, of introductions drives colonization success in a novel environment. *Ecol. Evol.* 8:8043–54
82. Kumar H, Panigrahi M, Chhotaray S, Bhanuprakash V, Shandilya R, et al. 2018. Red flour beetle (*Tribolium castaneum*): from population genetics to functional genomics. *Vet. World* 11:1043–46
83. Legault G, Bitters ME, Hastings A, Melbourne BA. 2020. Interspecific competition slows range expansion and shapes range boundaries. *PNAS* 117:26854–60
84. Lewis DL, DeCamillis M, Bennett RL. 2000. Distinct roles of the homeotic genes *Ubx* and *abd-A* in beetle embryonic abdominal appendage development. *PNAS* 97:4504–9
85. Linz DM, Tomoyasu Y. 2015. RNAi screening of developmental toolkit genes: a search for novel wing genes in the red flour beetle, *Tribolium castaneum*. *Dev. Genes Evol.* 225:11–22
86. Linz DM, Tomoyasu Y. 2018. Dual evolutionary origin of insect wings supported by an investigation of the abdominal wing serial homologs in *Tribolium*. *PNAS* 115:E658–67
87. Lorenzen MD, Gnirke A, Margolis J, Barnes J, Campbell M, et al. 2008. The maternal-effect, selfish genetic element Medea is associated with a composite Tc1 transposon. *PNAS* 105:10085–89
88. Lu Y, Beeman RW, Campbell JF, Park Y, Aikins MJ, et al. 2011. Anatomical localization and stereoisomeric composition of *Tribolium castaneum* aggregation pheromones. *Naturwissenschaften* 98:755–61

89. Lu Y, Park Y, Gao X, Zhang X, Yao J, et al. 2012. Cholinergic and non-cholinergic functions of two acetylcholinesterase genes revealed by gene-silencing in *Tribolium castaneum*. *Sci. Rep.* 2:288
90. Malekpour R, Rafter MA, Daglish GJ, Walter GH. 2016. Influence of phosphine resistance genes on flight propensity and resource location in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): the landscape for selection. *Biol. J. Linn. Soc.* 119:348–58
91. Matsumura K, Miyatake T. 2015. Differences in attack avoidance and mating success between strains artificially selected for dispersal distance in *Tribolium castaneum*. *PLOS ONE* 10:e0127042
92. Matsumura K, Miyatake T. 2019. Lines selected for different durations of tonic immobility have different leg lengths in the red flour beetle *Tribolium castaneum*. *Behaviour* 157:17–31
93. Matsumura K, Sasaki K, Miyatake T. 2016. Correlated responses in death-feigning behavior, activity, and brain biogenic amine expression in red flour beetle *Tribolium castaneum* strains selected for walking distance. *J. Ethol.* 34:97–105
94. Mertz DB. 1972. *Tribolium* model and the mathematics of population growth. *Annu. Rev. Ecol. Syst.* 3:51–78
95. Michalczyk L, Millard AL, Martin OY, Lumley AJ, Emerson BC, et al. 2011. Inbreeding promotes female promiscuity. *Science* 333:1739–42
96. Milutinovic B, Peuss R, Ferro K, Kurtz J. 2016. Immune priming in arthropods: an update focusing on the red flour beetle. *Zoology* 119:254–61
97. Miyatake T, Katayama K, Takeda Y, Nakashima A, Sugita A, Mizumoto M. 2004. Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. *Proc. R. Soc. B* 271:2293–96
98. Morrison WR, Wilkins RV, Gerken AR, Scheff DS, Zhu KY, et al. 2018. Mobility of adult *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) after exposure to long-lasting insecticide-incorporated netting. *J. Econ. Entomol.* 111:2443–53
99. Nayak MK, Daglish GJ, Phillips TW, Ebert PR. 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. *Annu. Rev. Entomol.* 65:333–50
100. Noh MY, Kramer KJ, Muthukrishnan S, Beeman RW, Kanost MR, Arakane Y. 2015. Loss of function of the *yellow-e* gene causes dehydration-induced mortality of adult *Tribolium castaneum*. *Dev. Biol.* 399:315–24
101. Noh MY, Kramer KJ, Muthukrishnan S, Kanost MR, Beeman RW, Arakane Y. 2014. Two major cuticular proteins are required for assembly of horizontal laminae and vertical pore canals in rigid cuticle of *Tribolium castaneum*. *Insect Biochem. Mol. Biol.* 53:22–29
102. Noh MY, Muthukrishnan S, Kramer KJ, Arakane Y. 2018. A chitinase with two catalytic domains is required for organization of the cuticular extracellular matrix of a beetle. *PLOS Genet.* 14:e1007307
103. Oppert B, Guedes RN, Aikins MJ, Perkin L, Chen Z, et al. 2015. Genes related to mitochondrial functions are differentially expressed in phosphine-resistant and -susceptible *Tribolium castaneum*. *BMC Genom.* 16:968
104. Oppert B, Perkin L. 2019. RNAiSeq: how to see the big picture. *Front. Microbiol.* 10:2570
105. Pai A, Bernasconi G. 2008. Polyandry and female control: the red flour beetle *Tribolium castaneum* as a case study. *J. Exp. Zool. B* 310:148–59
106. Pai A, Yan G. 2020. Long-term study of female multiple mating indicates direct benefits in *Tribolium castaneum*. *Entomol. Exp. Appl.* 168:398–406
107. Park T. 1962. Beetles, competition and populations: An intricate ecological phenomenon is brought into the laboratory and studied as an experimental model. *Science* 138:1369–75
108. Park Y, Aikins J, Wang LJ, Beeman RW, Oppert B, et al. 2008. Analysis of transcriptome data in the red flour beetle, *Tribolium castaneum*. *Insect Biochem. Mol. Biol.* 38:380–86
109. Perkin LC, Oppert B. 2019. Gene expression in *Tribolium castaneum* life stages: identifying a species-specific target for pest control applications. *PeerJ* 7:e6946
110. Pimentel MAG, Faroni LRDA, Tótola MR, Guedes RNC. 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. *Pest Manag. Sci.* 63:876–81
111. Pointer MD, Gage MJG, Spurgin LG. 2021. *Tribolium* beetles as a model system in evolution and ecology. *Heredity* 126:869–83

115. Describes the genome of *T. castaneum* and its utilization as a model species in genomics of economically important insect species.

122. Illustrates the loci that are related to DLD in phosphine resistance in *T. castaneum*.

134. Demonstrates that there are asymmetries in correlating insecticidal applications with population rebounds in *T. castaneum*.

112. Prühs R, Beermann A, Schröder R. 2017. The roles of the Wnt-antagonists axin and Lrp4 during embryogenesis of the red flour beetle *Tribolium castaneum*. *J. Dev. Biol.* 5:10
113. Rafter MA, McCulloch GA, Daglish GJ, Walter GH. 2017. Progression of phosphine resistance in susceptible *Tribolium castaneum* (Herbst) populations under different immigration regimes and selection pressures. *Evol. Appl.* 10:907–18
114. Rajendran S. 2000. Inhibition of hatching of *Tribolium castaneum* by phosphine. *J. Stored Prod. Res.* 36:101–6
115. Richards S, Gibbs RA, Weinstock GM, Brown SJ, Denell R, et al. 2008. The genome of the model beetle and pest *Tribolium castaneum*. *Nature* 452:949–55
116. Ridley AW, Hereward JP, Daglish GJ, Raghu S, Collins PJ, Walter GH. 2011. The spatiotemporal dynamics of *Tribolium castaneum* (Herbst): adult flight and gene flow. *Mol. Ecol.* 20:1635–46
117. Romero SA, Campbell JF, Nechols JR, With KA. 2009. Movement behavior in response to landscape structure: the role of functional grain. *Landscape Ecol.* 24:39–51
118. Romero SA, Campbell JF, Nechols JR, With KA. 2010. Movement behavior of red flour beetle: response to habitat cues and patch boundaries. *Environ. Entomol.* 39:919–29
119. Rösner J, Wellmeyer B, Merzendorfer H. 2020. *Tribolium castaneum*: a model for investigating the mode of action of insecticides and mechanisms of resistance. *Curr. Pharm. Des.* 26:3554–68
120. Ruckman SN, Blackmon H. 2020. The march of the beetles: Epistatic components dominate divergence in dispersal tendency in *Tribolium castaneum*. *J. Hered.* 111:498–505
121. Sang M, Li C, Wu W, Li B. 2016. Identification and evolution of two insulin receptor genes involved in *Tribolium castaneum* development and reproduction. *Gene* 585:196–204
122. Schlipalius DI, Valmas N, Tuck AG, Jagadeesan R, Ma L, et al. 2012. A core metabolic enzyme mediates resistance to phosphine gas. *Science* 338:807–10
123. Schmitt-Engel C, Schultheis D, Schwirz J, Ströhlein N, Troelener N, et al. 2015. The iBeetle large-scale RNAi screen reveals gene functions for insect development and physiology. *Nat. Commun.* 6:7822
124. Schultheis D, Weißkopf M, Schaub C, Ansari S, Dao VA, et al. 2019. A large scale systemic RNAi screen in the red flour beetle *Tribolium castaneum* identifies novel genes involved in insect muscle development. *G3* 9:1009–26
125. Semeao AA, Campbell JF, Whitworth RJ, Sloderbeck PE. 2013. Movement of *Tribolium castaneum* within a flour mill. *J. Stored Prod. Res.* 54:17–22
126. Shukla JN, Palli SR. 2013. *Tribolium castaneum* Transformer-2 regulates sex determination and development in both males and females. *Insect Biochem. Mol. Biol.* 43:1125–32
127. Smith FW, Jockusch EL. 2014. Hox genes require homothorax and extradenticle for body wall identity specification but not for appendage identity specification during metamorphosis of *Tribolium castaneum*. *Dev. Biol.* 395:182–97
128. Sokoloff A. 1966. *The Genetics of Tribolium and Related Species*. New York: Academic
129. Sokoloff A. 1972–1977. *The Biology of Tribolium with Special Emphasis on Genetic Aspects*, Vols. 1–3. Oxford, UK: Clarendon Press/Oxford Univ. Press
130. Soliman MH. 1987. Aging and parental age effects in *Tribolium*. *Arch. Gerontol. Geriatr.* 6:43–60
131. Stappert D, Frey N, von Levetzow C, Roth S. 2016. Genome-wide identification of *Tribolium* dorsoventral patterning genes. *Development* 143:2443–54
132. Szűcs M, Vahsen ML, Melbourne BA, Hoover C, Weiss-Lehman C, Hufbauer RA. 2017. Rapid adaptive evolution in novel environments acts as an architect of population range expansion. *PNAS* 114:13501–6
133. Thomson MS. 2014. A selfish gene chastened: *Tribolium castaneum* Medea M4 is silenced by a complementary gene. *Genetica* 142:161–67
134. Toews MD, Arthur FH, Campbell JF. 2009. Monitoring *Tribolium castaneum* (Herbst) in pilot-scale warehouses treated with b-cyfluthrin: Are residual insecticides and trapping compatible? *Bull. Entomol. Res.* 99:121–29
135. Tomoyasu Y, Miller SC, Tomita S, Schoppmeier M, Grossmann D, Bucher G. 2008. Exploring systemic RNA interference in insects: a genome-wide survey for RNAi genes in *Tribolium*. *Genome Biol.* 9:R10
136. Tong XL, Fu MY, Chen P, Chen L, Xiang ZH, et al. 2017. Ultrabithorax and abdominal-A specify the abdominal appendage in a dosage-dependent manner in silkworm, *Bombyx mori*. *Heredity* 118:578–84

137. Trebels B, Dippel S, Schaaf M, Balakrishnan K, Wimmer EA, Schachtner J. 2020. Adult neurogenesis in the mushroom bodies of red flour beetles (*Tribolium castaneum*, Herbst) is influenced by the olfactory environment. *Sci. Rep.* 10:1090
138. Ulrich J, Dao VA, Majumdar U, Schmitt-Engel C, Schwirz J, et al. 2015. Large scale RNAi screen in *Tribolium* reveals novel target genes for pest control and the proteasome as prime target. *BMC Genom.* 16:674
139. Vahsen ML, Shea K, Hovis CL, Teller BJ, Hufbauer RA. 2018. Prior adaptation, diversity, and introduction frequency mediate the positive relationship between propagule pressure and the initial success of founding populations. *Biol. Invasions* 20:2451–59
140. Vilcinskas A. 2016. The role of epigenetics in host-parasite coevolution: lessons from the model host insects *Galleria mellonella* and *Tribolium castaneum*. *Zoology* 119:273–80
141. Wang K, Liu M, Wang Y, Song W, Tang P. 2020. Identification and functional analysis of cytochrome P450 *CYP346* family genes associated with phosphine resistance in *Tribolium castaneum*. *Pestic. Biochem. Physiol.* 168:104622
142. Wang L, Wang S, Li Y, Paradesi MS, Brown SJ. 2007. BeetleBase: the model organism database for *Tribolium castaneum*. *Nucleic Acids Res.* 35:D476–79
143. Weiss-Lehman C, Hufbauer RA, Melbourne BA. 2017. Rapid trait evolution drives increased speed and variance in experimental range expansions. *Nat. Commun.* 8:14303
144. Wijayarathne LKW, Arthur FH, Whyard S. 2018. Methoprene and control of stored-product insects. *J. Stored Prod. Res.* 76:161–69
145. Xiao D, Gao X, Xu J, Liang X, Li Q, et al. 2015. Clathrin-dependent endocytosis plays a predominant role in cellular uptake of double-stranded RNA in the red flour beetle. *Insect Biochem. Mol. Biol.* 60:68–77
146. Zhu F, Parthasarathy R, Bai H, Woithe K, Kaussmann M, et al. 2010. A brain-specific cytochrome P450 responsible for the majority of deltamethrin resistance in the QTC279 strain of *Tribolium castaneum*. *PNAS* 107:8557–62

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137. Demonstrates that odor environment influences proliferation of Kenyon cells in the brain of *T. castaneum*.

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