Rice Weevils and Maize Weevils (Coleoptera: Curculionidae) Respond Differently to Disturbance of Stored Grain

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ABSTRACT Rice weevils, *Sitophilus oryzae* (L.), and maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), are important pests of stored grain. We have observed that rice weevil adults have a greater propensity than maize weevils to climb upward after physical disturbance of stored grain. Our objective was to quantify this climbing behavior in both species under a variety of environmental conditions to assess whether our anecdotal observations were correct. We confirmed that, after a disturbance, significantly more rice weevils climb than maize weevils when reared on fresh wheat, *Triticum aestivum* L., and corn, *Zea mays* L., and at relatively low and high population density. This greater climbing tendency is apparently exclusively due to *S. oryzae* males, which climb significantly more often than females. This work not only elucidates the divergent evolutionary histories of these sibling species but also may provide useful information for the monitoring and control of these stored grain pests.

KEY WORDS *Sitophilus oryzae*, *Sitophilus zeamais*, stored grain insects, negative geotaxis

Rice weevils, *Sitophilus oryzae* (L.), and maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), are cosmopolitan pests of stored grains. The life cycle of both species is very similar: adult females oviposit within a grain kernel, larvae and pupae spend their entire immature lifetime within the kernel, and adults emerge from the kernel and are capable of aerial dispersal (Longstaff 1981). Rice and maize weevils both exclusively consume seed endosperm, which is starch rich, but often vitamin and amino acid deficient. They are able to specialize on this narrow diet because they harbor obligate, maternally inherited bacterial symbionts that biosynthesize the scarce nutrients (Heddi 2003). We have been studying these *Sitophilus* spp. endosymbionts for several years (Dougherty and Plague 2008, Plague et al. 2008), which generally entails extracting larvae from grain kernels and dissecting bacteriomes (specialized host cells that house symbionts) to isolate the bacteria. In this time, we have noticed that *S. oryzae* adults consistently seem to climb much more after a physical disturbance of their grain store than *S. zeamais* adults. Stored grain insects, including *Sitophilus* spp., commonly exhibit negative geotaxis after a disturbance (Surtees 1963, Arbogast and Throne 1997), which may simply be an excited response to the disturbance (Smereka and Hodson 1959). If so, and if our anecdotal observations are correct, then *S. oryzae* is more excitable after a disturbance than *S. zeamais*, which is potentially an interesting behavioral difference between these ecologically similar sibling species. The objective of this study was to assess whether *S. oryzae* and *S. zeamais* adults do indeed exhibit differential climbing behaviors after a disturbance of their grain store.

Materials and Methods

To assess the climbing behaviors of *S. oryzae* and *S. zeamais* adults after a physical disturbance, we conducted behavioral assays under a variety of environmental conditions. All assays were performed at room temperature in small glass jars (6 cm in diameter by 9.2 cm in height) that contained 25 g of wheat, *Triticum aestivum* L., or corn, *Zea mays* L. (or an equivalent volume of spent grain; see below). The jars had a thin wooden climbing platform inserted vertically through the lid that reached ≈1.5 cm into the grain (the climbing surface below the lid was 8.3 by 3.3 by 0.2 cm). The circumference of each jar was lined with Fluon (BioQuip, Rancho Dominguez, CA) immediately above the grain to prevent weevils from climbing to the lid from the sides of the jar and immediately below the lid to prevent weevils from climbing down the sides from the lid. The *S. oryzae* and *S. zeamais* individuals were from cultures that have been maintained in the laboratory for ≥7 yr. Apart from the relatively high population density experiment (see below), all assays were performed with 100 *S. oryzae* or *S. zeamais* individuals per jar. Individual weevils were used in only

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one climbing assay. After allowing weevils to acclimate for ≥12 h after placement in an observation jar, we disturbed the jar by quickly inverting it clockwise 180° then counterclockwise 180° three successive times. After 30 min, we removed the lid with the attached climbing platform and quantified the number of weevils on the lid and the climbing platform (in preliminary studies, 30 min was the maximum time that weevils could climb up the platform and lid before beginning to displace one another due to high density).

We compared the climbing behavior of *S. oryzae* and *S. zeamais* individuals under four experimental conditions: 1) fresh preferred grain (i.e., *S. oryzae* on wheat and *S. zeamais* on corn; these are the grains on which each laboratory culture is reared); 2) fresh preferred grain, relatively high population density ($n = 200$ weevils per jar); 3) spent preferred grain (i.e., grain from the laboratory cultures; as such, this had decreased nutritional quality); and 4) fresh switched grain (i.e., *S. oryzae* on corn and *S. zeamais* on wheat). For each species, we performed 12 replicate assays of each experiment. For each experiment, we compared the proportion (arcsine transformed; Sokal and Rohlf 1995) of *S. oryzae* and *S. zeamais* individuals climbing from the grain store by using a two-tailed *t*-test.

In addition to testing different environmental conditions, we assessed whether *S. oryzae* or *S. zeamais* exhibit sexual differences in climbing propensity. In short, we sexed all individuals from five replicate assays of each species from the first climbing behavior experiment described above. After each assay, we preserved the climbers and nonclimbers separately in 70% ethanol, and then sexed each individual based on genitalia morphology (Halstead 1963). For each species, we compared the proportion (arcsine transformed) of climbing males and females by using a two-tailed *t*-test.

We also investigated whether age is correlated to climbing propensity in *S. oryzae* and *S. zeamais.* For this analysis, we first removed all *S. oryzae* and *S. zeamais* adults from samples of infested wheat and corn, respectively. Then, every day for 30 d, we removed all newly emerged adults from the infested wheat and corn and marked eight or more individuals of each species with a unique combination of color paint spots on their elytra (we used nail polish; these marks did not seem to hinder the weevils' mobility). On day 33, we performed two climbing assays for each species (as described above) by using fresh preferred grain and a mixture of individuals of all ages (for *S. oryzae* assays, mean = 3.3 individuals per age (in days), $n = 99$ and 100 total individuals; for *S. zeamais* assays, mean = 3.4 individuals per age, $n = 100$ and 103 total individuals). We quantified the proportion of weevils of each age that climbed and tested whether the linear regression between age and proportion climbing (arcsine transformed) deviates significantly from zero.

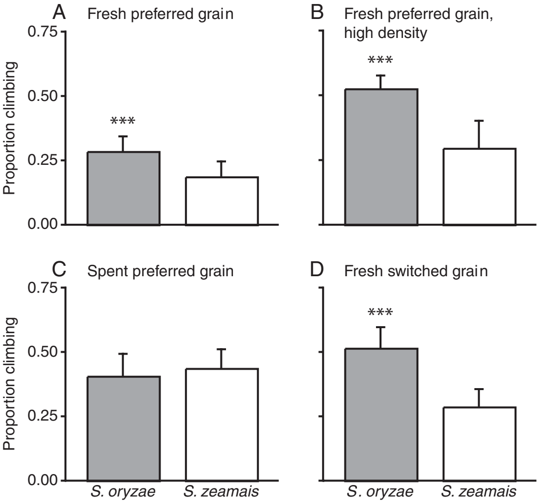

Fig. 1. Proportions of *S. oryzae* and *S. zeamais* adults climbing after a physical disturbance, when reared on fresh preferred grain (i.e., *S. oryzae* on wheat, *S. zeamais* on corn) (A); fresh preferred grain, high population density ($n = 200$ weevils, two-fold more than the other treatments) (B); spent preferred grain (i.e., grain from laboratory cultures) (C); and fresh switched grain (i.e., *S. oryzae* on corn, *S. zeamais* on wheat) (D). The error bars represent 1 SD, and asterisks indicate statistically significant differences (***, $P \leq 0.001$).

Results and Discussion

We confirmed that at both relatively low and high infestation densities on fresh grain, a significantly greater proportion of *S. oryzae* adults climb after a disturbance than *S. zeamais* adults (low density: $t = 4.31$, df = 22, $P < 0.001$; high density: $t = 6.42$, df = 22, $P < 0.001$) (Fig. 1A and B). However, this behavioral difference does not extend to spent grain, where both species are equally likely to climb ($t = -0.92$, df = 22, $P = 0.37$) (Fig. 1C). *S. oryzae*'s greater propensity to climb on fresh grain (or conversely, *S. zeamais*' greater reluctance to climb) is not simply a response to the grain on which they are reared, because a significantly higher proportion of them climb than *S. zeamais* in assays performed on the other's preferred grain ($t = 7.23$, df = 22, $P < 0.001$) (Fig. 1D). Therefore, on fresh grain, *S. oryzae* adults have a greater innate tendency to climb after a disturbance than *S. zeamais* adults. This greater climbing tendency is apparently exclusively due to *S. oryzae* males (Fig. 2A), which climb significantly more often than females ($t = 5.05$, df = 8, $P < 0.001$). In fact, the proportion of female *S. oryzae* climbers is not significantly different than the proportion of *S. zeamais* male ($t = 1.05$, df = 8, $P = 0.33$) or female climbers ($t = 0.04$, df = 8, $P = 0.97$) (compare Fig. 2A and B). *S. zeamais,* however, exhibits no climbing difference between sexes ($t = -1.04$, df = 8, $P = 0.33$) (Fig. 2B). Also, *S. zeamais* exhibits a weak but statistically significant negative correlation between age and climbing propensity after a disturbance ($F = 4.229$, df = 1, 58, $P = 0.044$, $r^2 = 0.068$) (Fig. 3B). However, *S. oryzae* exhibits no such correlation ($F = 0.616$, df = 1, 54, $P = 0.436$, $r^2 = 0.011$) (Fig. 3A).

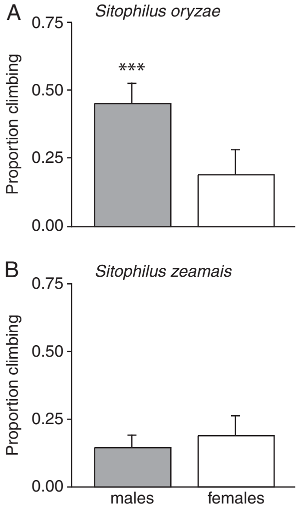

Fig. 2. Proportions of *S. oryzae* and *S. zeamais* males and females climbing after a physical disturbance, when reared on fresh preferred grain. The error bars represent 1 SD, and the asterisk indicates a statistically significant difference (***, $P \leq 0.001$).

Many stored grain insects exhibit negative geotaxis after a disturbance (Arbogast and Throne 1997). Therefore, this behavior apparently has evolved multiple times in independent lineages. Phenotypic convergence is often strong evidence for adaptive evolution (Futuyma 1998), so negative geotaxis is probably frequently an adaptive response to disturbance in stored grain insects. We suspect that this also may be true for *S. oryzae* and *S. zeamais.* Because both species exhibit this climbing behavior, it is likely that their last common ancestor ≈20 million yr ago (O'Meara 2001) also exhibited this behavior. Because this ancestor predates the origin of hominids (Glazko and Nei 2003), it did not infest human grain stores. Instead, we suspect that it may have primarily infested the seed caches of birds and rodents (Davis and Sloop 1934, Linsley 1944). If so, then the negative geotaxis exhibited by some *Sitophilus* spp. individuals may have evolved as a response to these owners returning to their grain store, i.e., this was the original disturbance. Consequently, this climbing behavior may have been an adaptive reaction to avoid predation (intentional or incidental), or as the first step in dispersing to a new grain store because the persistence of its present store was suddenly in doubt. When owners returned, adult weevils had to decide whether to stay or leave the cache. From an evolutionary perspective, this decision, or "Darwinian algorithm" (Cosmides and Tooby 1987), presumably would have been driven by a cost–benefit analysis, comparing the quality of the current cache to the costs and likelihood of finding a new cache. Our finding that significantly more *S. oryzae* and *S. zeamais* adults climb on spent than fresh grain supports this idea (*S. oryzae:* $t = 4.14$, df = 22, $P < 0.001$; *S. zeamais:* $t = 9.25$, df = 22, $P < 0.001$; compare Fig. 1A and C); i.e., adults of both species seem to be more willing to abandon low-quality than high-quality

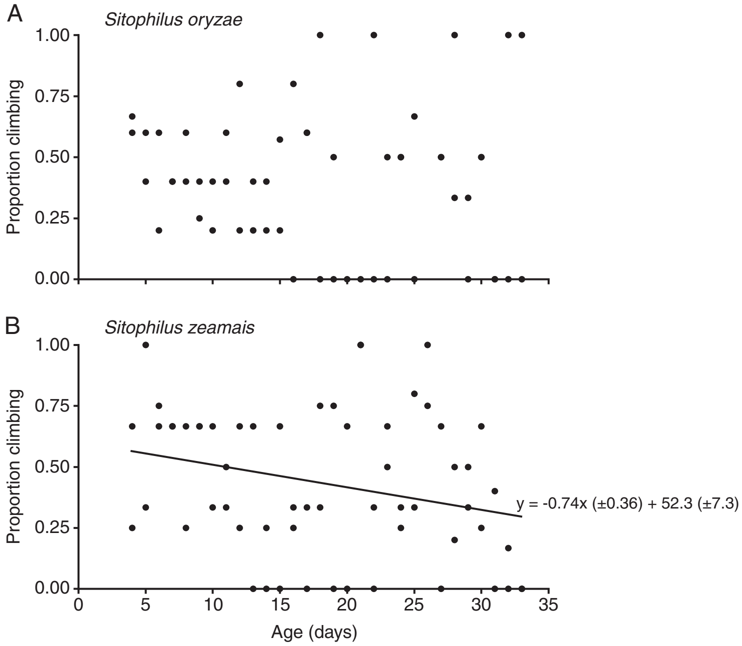

Fig. 3. Relationship between the proportion of weevils climbing after a physical disturbance and adult age (in days). (A) For *S. oryzae*, the relationship is not statistically significant ($P = 0.436$, $r^2 = 0.011$). (B) For *S. zeamais*, the relationship is significant ($P = 0.044$, $r^2 = 0.068$); the regression analysis used the arcsine-transformed proportions; the parenthetical values in the regression equation are ±1 SE.

caches. Furthermore, the desire to seek a new grain store may partly explain the differential climbing propensity between *S. oryzae* and *S. zeamais* after a disturbance (Fig. 1). Specifically, *S. oryzae* are poor fliers compared with *S. zeamais* (Kiritani 1959 [as cited in Longstaff 1981], Vásquez-Castro et al. 2009) and as such may require more time to escape hazardous conditions (Perttunen and Killström 1971). However, this does not explain the climbing difference between *S. oryzae* males and females (Fig. 2A), because dispersal presumably would be equally advantageous for both sexes, unless females are better fliers than males (Likhayo and Hodges 2000). In human-owned grain stores, which *S. oryzae* and *S. zeamais* probably predominantly infest today, disturbance may continue to signify imminent grain consumption. As such, negative geotaxis may still be an advantageous behavior and not simply a relic of past selective pressures (Kriska et al. 1998).

In addition to elucidating the ecological and evolutionary histories of *S. oryzae* and *S. zeamais* weevils, this work also may be useful in population monitoring and control of these stored grain pests. For example, because these weevils climb after a disturbance, treating the walls and ceilings of storage bins with contact insecticide, followed by periodic physical disturbance of the grain store, may be an effective control strategy, particularly for *S. oryzae* (Fig. 1). Applying the insecticide to the bin (Toews et al. 2003) instead of the grain itself (Getchell and Subramanyam 2008) may reduce pesticide exposure for the intended consumers. This strategy would preferentially target 1) male *S. oryzae* (Fig. 2A), which is not as ideal as targeting females in terms of limiting population growth and grain damage (both *S. zeamais* sexes would be equally targeted; Fig. 2B); and 2) young *S. zeamais* individuals (Fig. 3B), which is ideal for population control (all *S. oryzae* ages would be equally targeted; Fig. 3A), although this age preference is weak. Despite these limitations, it may be worthwhile to investigate whether this strategy could provide enough control to keep *Sitophilus* spp. populations below economic threshold levels. Although it may be difficult to adequately disturb the relatively large grain stores common in the United States, this approach may be efficacious with the smaller stores prevalent in developing nations (Proctor 1994).

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References Cited

Arbogast, R. T., and J. E. Throne. 1997. Insect infestation of farm-stored maize in South Carolina: towards characterization of a habitat. J. Stored Prod. Res. 33: 187–198.

Cosmides, L., and J. Tooby. 1987. From evolution to behavior: evolutionary psychology as the missing link, pp. 277–306. *In* J. Dupré (ed.), The latest on the best: essays on evolution and optimality. MIT Press, Cambridge, MA.

Davis, A. C., and K. D. Sloop. 1934. Notes on some insects found in the burrows of the California ground squirrel in Orange County, California. Bull. Brooklyn Entomol. Soc. 29: 79–83.

Dougherty, K. M., and G. R. Plague. 2008. Transposable element loads in a bacterial symbiont of weevils are extremely variable. Appl. Environ. Microbiol. 74: 7832–7834.

Futuyma, D. J. 1998. Evolutionary biology. Sinauer, Sunderland, MA.

Getchell, A. I., and B. Subramanyam. 2008. Immediate and delayed mortality of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) adults exposed to spinosad-treated commodities. J. Econ. Entomol. 101: 1022–1027.

Glazko, G. V., and M. Nei. 2003. Estimation of divergence times for major lineages of primate species. Mol. Biol. Evol. 20: 424–434.

Halstead, D.G.H. 1963. External sex differences in stored-products Coleoptera. Bull. Entomol. Res. 54: 119–134.

Heddi, A. 2003. Endosymbiosis in the weevil of the genus *Sitophilus:* genetic, physiological, and molecular interactions among associated genomes, pp. 67–82. *In* K. Bourtzis and T. A. Miller (eds.), Insect symbiosis. CRC, Boca Raton, FL.

Kiritani, K. 1959. Flying ability and some of the characters associated with it in *Calandra.* Jpn. J. Ecol. 9: 69–74.

Kriska, G., G. Horváth, and S. Andrikovics. 1998. Why do mayflies lay their eggs *en masse* on dry asphalt roads? Water-imitating polarized light reflected from asphalt attracts Ephemeroptera. J. Exp. Biol. 201: 2273–2286.

Likhayo, P. W., and R. J. Hodges. 2000. Field monitoring *Sitophilus zeamais* and *Sitophilus oryzae* (Coleoptera: Curculionidae) using refuge and flight traps baited with synthetic pheromone and cracked wheat. J. Stored Prod. Res. 36: 341–353.

Linsley, E. G. 1944. Natural sources, habitats, and reservoirs of insects associated with stored food products. Hilgardia 16: 187–224.

Longstaff, B. C. 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. Prot. Ecol. 2: 83–130.

O'Meara, B. 2001. Bacterial symbiosis and plant host use evolution in Dryophthorinae (Coleoptera, Curculionidae): a phylogenetic study using parsimony and Bayesian analysis. B.A. thesis, Harvard University, Cambridge, MA.

Perttunen, V., and K. M. Killström. 1971. Effect of desiccation on the light reactions of *Calandra granaria* L. and *Calandra oryzae* L. (Col., Curculionidae). Ann. Entomol. Fenn. 37: 147–154.

Plague, G. R., H. E. Dunbar, P. L. Tran, and N. A. Moran. 2008. Extensive proliferation of transposable elements in heritable bacterial symbionts. J. Bacteriol. 190: 777–779.

Proctor, D. L. 1994. Grain storage techniques: evolution and trends in developing countries. FAO Agricultural Services Bulletin No. 109. Food and Agriculture Organization, Rome, Italy.

Smereka, E. P., and A. C. Hodson. 1959. Some humidity and light reactions of the granary weevil, *Sitophilus granarius*

(L.) (Coleoptera: Curculionidae). Can. Entomol. 91: 784–797.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. W. H. Freeman, New York.

Surtees, G. 1963. Laboratory studies on dispersion behaviour of adult beetles in grain. I. The grain weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae). Bull. Entomol. Res. 54: 147–159.

Toews, M. D., B. Subramanyam, and J. M. Rowan. 2003. Knockdown and mortality of adults of eight species of stored-product beetles exposed to four surfaces treated with spinosad. J. Econ. Entomol. 96: 1967–1973.

Vásquez-Castro, J. A., G. C. de Baptista, L.R.P. Trevizan, and C. D. Gadanha. 2009. Flight activity of *Sitophilus oryzae* (L) and *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and its relationship with susceptibility to insecticides. Neotrop. Entomol. 38: 405–409.

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