

# Influence of Age, Mating Status, Sex, Quantity of Food, and Long-Term Food Deprivation on Red Flour Beetle (Coleoptera: Tenebrionidae) Flight Initiation

J. PEREZ-MENDOZA, J. F. CAMPBELL, AND J. E. THRONE<sup>1</sup>

USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan KS 66502

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**ABSTRACT** Effects of age, sex, presence or absence of food, mating status, quantity of food, and food deprivation on rate of and time of flight initiation of the red flour beetle, *Tribolium castaneum* (Herbst), were determined. Flight initiation declined with increasing age in both presence and absence of food. However, flight initiation was lower when food was present in the flight chambers than in the absence of food. In the presence of food, both mated and virgin beetles were equally likely to disperse by flight. However, in the absence of food, mated beetles initiated flight more readily than virgin individuals. Flight initiation was greatest when little or no food was present. The presence of varying quantities of food inside the flight chambers impacted the number of progeny produced by females before flight, but not the timing of flight. Rate of flight initiation was higher for beetles deprived of food for short periods of time compared with flight initiation of beetles with food in the flight chamber. Flight initiation decreased with increasing time without food. There were no differences in flight tendencies between males and females in the experiments reported here. Our results suggest that *T. castaneum* uses flight as a mechanism to disperse to new environments during almost any part of their life span and that this type of dispersion does not fit with the model of the so-called true migratory species that involves an “oogenesis-flight syndrome.”

**KEY WORDS** flight initiation, mating status, presence or absence of food, starvation, *Tribolium castaneum*

Landscapes are spatially heterogeneous, and resources required by insects (e.g., habitat, food, shelter, and mates) are patchily distributed within the landscape (Wiens 2001). This heterogeneity can influence insect movement patterns, including the tendency of insects to initiate flight (Johnson et al. 1992). Dispersal capacity by flight is an important adaptive trait in insects that can determine their success or failure in finding new resource patches (Johnson 1969). Stored-product insects exploit spatially heterogeneous landscapes with spatially separated food patches that vary considerably in size, quality, and persistence (Campbell et al. 2006), and efficient and accurate mechanisms for search and assessment are crucial for an individual's fitness in this type of landscape (Bell 1990). Many stored-product insects use a combination of walking and flight behavior during the process of dispersal and colonization (e.g., Ziegler 1976).

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a worldwide pest of stored grain and of facilities used for the processing (e.g., flour mills, bakeries, pasta plants, and pet food manufacturing facilities) or storage of grain or grain-

based products (e.g., retail stores, warehouses, distribution centers, and home pantries) (Surtees 1963, Hagstrum and Gilbert 1976, Boon and Ho 1988, Vela-Coiffier et al. 1997, Roesli et al. 2003, Campbell and Arbogast 2004, Toews et al. 2009, Campbell et al. 2010). The red flour beetle is well adapted to heterogeneous environments of spatially separated food patches, and it readily disperses from food patches and visits multiple resource patches over its lifetime (Naylor 1961; Ziegler 1977, 1978; Campbell and Hagstrum 2002; Campbell and Runnion 2003). Dispersing females distribute their eggs among different food patches, but they produce fewer progeny in food patches of lower quality (Ziegler 1976, Campbell and Runnion 2003). This ability has contributed to their pest status. Research has focused on their dispersal by walking, but adults also disperse by flight.

Adult red flour beetles have long been recognized to fly readily under laboratory conditions if temperatures are warm enough (generally over 25°C) (Zirkle et al. 1988, Cox et al. 2007), and they also have been observed flying in and around food storage facilities (Jones 1967, Giles 1969, Sinclair and Haddrell 1985, Boon and Ho 1988, Throne and Cline 1994, Ho and Boon 1995). However, we are not aware of any studies

<sup>1</sup> Corresponding author, e-mail: james.throne@ars.usda.gov.

that have evaluated the factors that influence red flour beetle flight initiation behavior, other than our previous work, even though this information is needed to understand its role in dispersal and colonization behavior in food processing and storage facilities. Flight initiation behavior of the stored-product pests the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), has been extensively studied (Barrer et al. 1993, Aslam et al. 1994, Dowdy 1994, Fadamiro et al. 1996, Scholz et al. 1997, Perez-Mendoza et al. 1999) and appears to be greater in newly emerged adults and from food patches of declining quality. In a previous study, we evaluated the influence of rearing density, short-term chronological age, sex, presence or absence of food, short-term food deprivation, and number and sex of companion beetles on flight initiation of male and female red flour beetles (Perez-Mendoza et al. 2011). Unlike the lesser and larger grain borers (Barrer et al. 1993, Dowdy 1994, Fadamiro et al. 1996, Perez-Mendoza et al. 1999), red flour beetle flight initiation behavior was not influenced by rearing density, adult density, age, or duration of food deprivation. Similar to results for the grain borers (Dowdy 1994, Fadamiro 1997), sex of the adults and presence or absence of food inside flight chambers did not affect flight initiation of red flour beetles. However, both males and females tended to initiate flight less readily when they were in the company of individuals of the opposite sex. In the current study, we tested the effects of adult mating status, the presence or absence of food, the quantity of food, extended periods of food deprivation (up to 20 d vs. up to 5 d in our previous study), and long-term chronological adult age (up to 215 d vs. up to 20 d in our previous study) on flight initiation in red flour beetles. We also determined the number of progeny produced by females that either flew or did not fly during a 3-d flight initiation test.

### Materials and Methods

**Culture Methods.** The *T. castaneum* colony used in these studies was originally obtained from a flour mill and had been in culture for 7 mo at the time of these experiments. Unless otherwise indicated, adults used in the experiments were obtained from stock cultures established by introducing 100 unsexed, 2- to 4-wk-old adults into 200 g of diet containing 95 parts wheat flour and five parts brewer's yeast in quart jars, and then removing all adults after 3 d. All cultures were maintained at  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 L:D photoperiod.

**Flight Bioassay.** Flight initiation was assayed in a flight chamber developed by Perez-Mendoza et al. (2011) consisting of the bottom half of a petri dish ( $60 \times 20$  mm) with the inner surface coated with sticky material (Sticky Stuff, Olson Products, Medina, OH) inverted over another uncoated petri dish bottom (base dish) of the same dimensions. The sides of the base dish were coated with Teflon PTFE 30 fluorocarbon resin (DuPont, Wilmington, DE) to pre-

vent insects from walking up the sides of the base dish. The base dish was furnished with a platform to facilitate flight initiation.

Individual adults were placed in the base dishes, and the base dishes were immediately covered with the coated petri dish. The assembled flight chambers were placed on plastic trays and held in the laboratory in the dark at room temperature ( $\approx 24^\circ\text{C}$ ) for 1 h. Then the trays containing the chambers and insects were transferred to an environmental chamber maintained at  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 L:D photoperiod, just after lights were turned on. The number of adults that initiated flight (adhered to the sticky bottom half petri dish) was recorded after 24, 48, and 72 h.

**Effect of Age, Sex, and Presence or Absence of Food on Flight Initiation.** To measure the impact of age (days after emergence from pupa) on flight initiation, emerged male and female adults from the stock cultures were maintained alive for different periods of time. Insect cultures were started by introducing 200 unsexed adults into 200 g of wheat flour + 5% yeast in 800 ml glass jars that had screen/filter paper lids. Jars were held at  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 L:D photoperiod. All founding adults were removed by sieving after 3 d. This rearing cycle was repeated weekly for 12 wk to produce adults of different ages. Immatures were reared at  $30^\circ\text{C}$  for rapid development, but the cultures were removed from the  $30^\circ\text{C}$  rearing chamber after adult emergence and transferred to another chamber maintained at  $20 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  RH, and 12:12 L:D photoperiod to prolong adult longevity so that we could obtain beetles from a broad range of ages for testing. Adults from each age group were transferred to new diet every 3 wk to avoid contamination from emerging progeny. Beetles were sexed by checking for the presence (males) or absence (females) of a subbasal setiferous puncture on the anterior femur (Halstead 1963).

Adult males and females that were 8, 34, 72, 103, 128, 165, 182, and 215 d postemergence from pupae were tested for flight initiation. We selected these age classes because our previous study showed no differences in flight initiation of young adults (1–4, 7–11, and 16–20-d-old; Perez-Mendoza et al. 2011). Thirty males or 30 females of each age were introduced individually into 30 flight chambers with 1 g of diet and 30 chambers without diet to assess flight initiation. A total of 960 insects were tested ( $n = 30$  for each age/sex/food category).

We also determined progeny production for individual parent females of different age classes. Because mortality of beetles after 150 d was high, we were unable to use beetles from the same rearing batch and with the same ages as in the flight test. Therefore, we used insects from another rearing batch in this test although the ages of the females were not exactly the same as the insects used in the flight test because of timing issues. We used the same rearing technique described above to obtain mated adults of different ages. Twenty mated females aged 14, 57, 82, 120, 152, 177, and 215 d old were placed individually into plastic vials ( $3.2 \times 8$  cm) containing 20 g of diet, and the vials

were sealed with snap-cap lids with 40-mesh screen covering a 1-cm diameter opening. Females were allowed to oviposit for 3 d at  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 L:D photoperiod, and then they were removed. Number of adult progeny produced by each female was determined by incubating the diet in each vial until adult emergence was completed ( $\approx 40$  d). There was no parental mortality during the oviposition period, and we did not determine immature mortality directly.

**Effect of Mating Status and Presence or Absence of Food on Flight Initiation.** To measure the effect of mating status on flight initiation, pupae were obtained from stock cultures and sexed based on size of the papillae (female papillae are larger than those of the male; Park 1934). Groups of 250 male and 250 female pupae were sexed and introduced in sets of 125, keeping sexes separate, in four 237-ml (one-half pint) glass jars containing 20 g of diet. One week after adult emergence, 125 virgin males and 125 virgin females, marked with a dot of white nail polish on their thorax for identification, were paired with a member of the opposite sex for 72 h in individual glass tubes ( $13 \times 100$  mm) that contained 1 g of diet, after which the adults were separated from each other and returned to their respective pint jars. Thus, we had one jar containing virgin males, one containing virgin females, one containing males that had the opportunity to mate, and one containing females that had the opportunity to mate. Four weeks later, 60 adults from each of the four groups (mated and unmated, males and females) were placed individually inside individual flight chambers with 1 g of diet and a similar set of beetles were placed individually in chambers without food ( $n = 480$  beetles) to assess flight initiation. Adults used in the flight tests were 39–42 d in age postemergence from pupae.

**Effect of Quantity of Food Inside Flight Chambers on Flight Initiation.** In this experiment, we tested the effect of presence of food in varying quantities inside the flight chambers on flight initiation of 30- to 32-d old mated male and female beetles from the stock cultures. Beetles were sexed by external sex differences (Halstead 1963). Male and female adults were placed individually inside individual flight chambers that contained 0, 0.01, 0.1, 0.3, 0.6, or 1.0 g of diet to assess flight initiation. Sixty male and 60 female beetles were tested for each food quantity treatment ( $n = 720$  insects). To determine if females laid eggs before initiating flight, diet from the flight chambers was held for 2 wk after the flight test. Number of progeny per female was determined by sieving, and the number of individuals produced were recorded and compared between females initiating or not initiating flight.

**Effect of Long-Term Food Deprivation on Flight Initiation.** Beetles used in this study were held without food for varying periods of time before testing, and their flight initiation abilities were compared with beetles provided with food. Male and female adults were obtained as described in the previous experiment. Food-deprived beetles were obtained by placing groups of 40 beetles (32- to 35-d-old) in petri dishes ( $60 \times 20$  mm) without food, while control

beetles were held in petri dishes ( $60 \times 20$  mm) with 20 g of diet. The dishes were covered with lids and placed in an environmental chamber at  $30 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 L:D photoperiod for 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 d. After these holding periods, food-deprived beetles were placed individually in flight chambers without food, and fed beetles were placed individually in flight chambers containing 0.2 g of diet. In a previous experiment, we determined that the presence of 0.2 g of diet inside the flight chamber did not affect flight initiation in this species (Perez-Mendoza et al. 2011). Thirty male and 30 female adults for each treatment combination were tested for flight initiation ( $n = 660$  beetles).

**Statistical Analysis.** Differences in proportion of tested individuals initiating flight during the first 72 h among different treatments were analyzed using the GLIMMIX procedure with the binary distribution and the Tukey-Kramer multiple range test (SAS Institute 2008). The General Linear Models (PROC GLM) procedure (SAS Institute 2008) and the Ryan-Einot-Gabriel-Welsh (REGWQ) multiple range test were used to determine if there were significant differences in number of progeny produced and time to initiate flight.

We usually used regression to examine differences among levels of treatments that had a quantitative independent variable. A number of different types of equations were fit to the data using TableCurve 2D (SYSTAT Software 1999). Selection of an equation to describe the data was based on the magnitude and pattern of residuals, lack of fit tests, and  $R^2$  values (Draper and Smith 1981). We also ensured that the shape of the curve was reasonable for describing the data.

## Results

**Effect of Age, Sex, and Presence or Absence of Food on Flight Initiation.** No beetles that were 182- or 215-d-old initiated flight during this experiment; therefore, those two ages were not included in the statistical analyses. Flight initiation varied with age ( $F = 14.8$ ;  $df = 5$ , 696;  $P < 0.01$ ) and presence or absence of food inside the flight chamber ( $F = 29.0$ ;  $df = 1$ , 696;  $P < 0.01$ ), but not with sex ( $F = 2.0$ ;  $df = 1$ , 696;  $P = 0.16$ ). The interactions between age and sex ( $F = 3.2$ ;  $df = 5$ , 696;  $P = 0.06$ ) and sex and food ( $F = 2.8$ ;  $df = 1$ , 696;  $P = 0.10$ ) were not significant, but the interaction between age and food ( $F = 11.6$ ;  $df = 5$ , 696;  $P < 0.01$ ) and the three way interaction (age-sex-food) ( $F = 3.5$ ;  $df = 5$ , 696;  $P < 0.01$ ) were significant. We then analyzed the data by presence or absence of food, but sex and the interaction of sex and age still were not significant whether or not food was present (results not shown). Therefore, we combined data for the sexes and reanalyzed using a two-way analysis of variance (ANOVA). After combining sexes, flight initiation varied with age (Fig. 1;  $F = 8.7$ ;  $df = 5$ , 348;  $P < 0.01$ ) and with presence or absence of food ( $F = 32.7$ ;  $df = 1$ , 348;  $P < 0.01$ ), and the interaction was not significant ( $F = 0.2$ ;  $df = 5$ , 348;  $P = 0.95$ ).

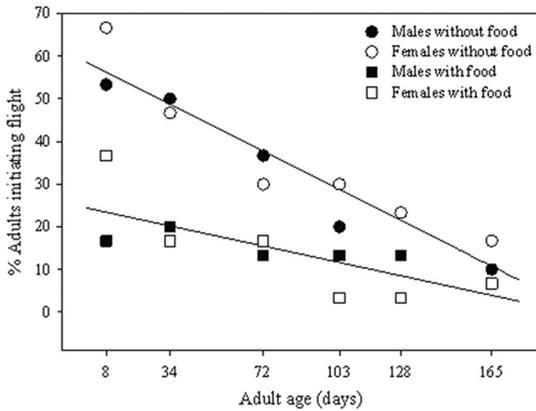


Fig. 1. Effect of age and presence or absence of food on flight initiation of male and female *T. castaneum*. The relationship between flight initiation (*y*) and age (*x*) was described by the following equations: with food,  $y = 24.45$  ( $SE = 3.3$ )  $- 0.124$  ( $0.03$ ), ( $R^2 = 0.59$ ;  $n = 12$ ); and without food,  $y = 58.55$  ( $2.9$ )  $- 0.289$  ( $0.03$ ), ( $R^2 = 0.91$ ;  $n = 12$ ).

Flight initiation of beetles declined with increasing age in both presence and absence of food. However, flight initiation was lower in beetles when food was present in the flight chambers than in the absence of food (Fig. 1).

The time of flight initiation varied with age ( $F = 2.6$ ;  $df = 5, 170$ ;  $P = 0.03$ ), but not with sex ( $F = 0.5$ ;  $df = 1, 170$ ;  $P = 0.49$ ) or presence or absence of food ( $F = 3.4$ ;  $df = 1, 170$ ;  $P = 0.07$ ). None of the interactions were significant: age-sex ( $F = 0.7$ ;  $df = 5, 170$ ;  $P = 0.65$ ), sex-food ( $F = 0.3$ ;  $df = 1, 170$ ;  $P = 0.60$ ), age-food ( $F = 2.2$ ;  $df = 5, 170$ ;  $P = 0.06$ ), and age-sex-food ( $F = 0.8$ ;  $df = 5, 170$ ;  $P = 0.58$ ). Although the overall analysis showed a significant impact of adult age on time of flight initiation, adult age treatments were not significantly different from each other using the REGWQ means separation test with an experiment-wise error

rate of 0.05. The mean time of flight initiation varied from  $1.6 \pm 0.14$  d in 34-d-old beetles to  $2.2 \pm 0.17$  d in 72-d-old beetles.

Progeny production varied with age ( $F = 13.3$ ;  $df = 6, 140$ ;  $P < 0.01$ ; Fig. 2). Maximum adult progeny production occurred in females from the ages of 57- to 120-d-old. Progeny production was reduced and similar in relatively young (14-d-old) and old females ( $\geq 152$ -d-old).

**Effect of Mating Status and Presence or Absence of Food on Flight Initiation.** Flight initiation varied with presence or absence of food inside the flight chambers ( $F = 83.9$ ;  $df = 1, 472$ ;  $P < 0.01$ ), but not with sex ( $F = 2.3$ ;  $df = 1, 472$ ;  $P = 0.13$ ) or mating status ( $F = 2.9$ ;  $df = 1, 472$ ;  $P = 0.09$ ). Most of the interactions were not significant: food-sex ( $F = 0.7$ ;  $df = 1, 472$ ;  $P = 0.39$ ), sex-mating status ( $F = 2.9$ ;  $df = 1, 472$ ;  $P = 0.09$ ), and food-sex-mating status ( $F = 0.3$ ;  $df = 1, 472$ ;  $P = 0.86$ ). However, the interaction between food and mating status ( $F = 9.2$ ;  $df = 1, 472$ ;  $P < 0.01$ ) was significant. We then analyzed the data by presence or absence of food, but sex and the interaction of sex and mating status still were not significant whether or not food was present (results not shown). Therefore, we combined data for the sexes and reanalyzed using a two-way ANOVA. After combining sexes, flight initiation did not vary with mating status ( $F = 2.6$ ;  $df = 1, 236$ ;  $P = 0.11$ ), but varied with presence or absence of food (Fig. 3;  $F = 85.0$ ;  $df = 1, 236$ ;  $P < 0.01$ ), and the interaction also was significant ( $F = 10.4$ ;  $df = 1, 236$ ;  $P < 0.01$ ). Thus, we reanalyzed the data by presence or absence of food within each mating status. Without food, mated beetles initiated flight more readily than virgin individuals (Fig. 3;  $F = 13.1$ ;  $df = 1, 118$ ;  $P < 0.01$ ). With food, flight initiation did not vary with mating status ( $F = 1.0$ ;  $df = 1, 118$ ;  $P = 0.28$ ).

The time of flight initiation varied with presence or absence of food ( $F = 25.1$ ;  $df = 1, 209$ ;  $P < 0.01$ ), but not with sex ( $F = 0.01$ ;  $df = 1, 209$ ;  $P = 0.94$ ) or mating

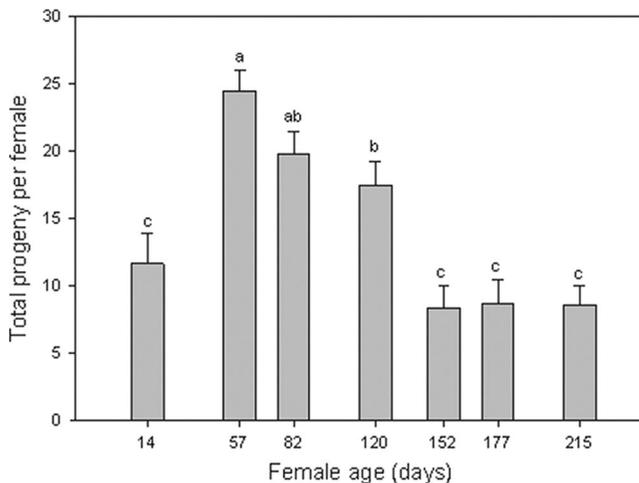


Fig. 2. Effect of age on progeny production of female *T. castaneum*. Different letters above bars indicate differences among means (Ryan-Einot-Gabriel-Welsh multiple range test).

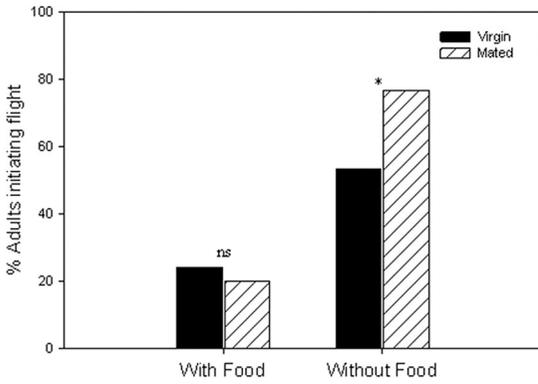


Fig. 3. Effect of presence or absence of food on flight initiation of virgin and mated male and female *T. castaneum*. “ns” indicates no difference between treatments, and “\*” indicates treatment responses differ (ANOVA).

status ( $F = 0.2$ ;  $df = 1, 209$ ;  $P = 0.69$ ). None of the interactions were significant: food-sex ( $F = 0.9$ ;  $df = 1, 209$ ;  $P = 0.35$ ), sex-mating status ( $F = 0.5$ ;  $df = 1, 209$ ;  $P = 0.47$ ), mating status-food ( $F = 0.5$ ;  $df = 1, 209$ ;  $P = 0.47$ ), and mating status-sex-food ( $F = 0.2$ ;  $df = 1, 209$ ;  $P = 0.70$ ). Mated and virgin adults without food initiated flight later ( $1.8 \pm 0.07$  d) than mated and virgin adults with food ( $1.2 \pm 0.06$  d).

**Effect of Quantity of Food Inside Flight Chambers on Flight Initiation.** Flight initiation varied with the amount of food inside the flight chamber ( $F = 9.0$ ;  $df = 5, 348$ ;  $P < 0.01$ ), but not with sex ( $F = 0.04$ ;  $df = 1, 348$ ;  $P = 0.85$ ) and the interaction also was not significant ( $F = 0.6$ ;  $df = 5, 348$ ;  $P = 0.71$ ). Therefore, we combined data for the sexes and described flight initiation as a function of quantity of food inside the flight chambers (Fig. 4A; Table 1). Flight initiation decreased as quantity of food increased.

Time of flight initiation did not vary with the amount of food inside the flight chamber ( $F = 1.9$ ;  $df = 5, 209$ ;  $P = 0.09$ ) or with sex ( $F = 0.2$ ;  $df = 1, 209$ ;  $P = 0.67$ ), and the interaction also was not significant ( $F = 0.8$ ;  $df = 5, 209$ ;  $P = 0.60$ ). The mean time of flight initiation in all treatments combined was  $1.4 \pm 0.07$  d.

The percentage of females (both females that did not fly + females that flew) that produced progeny increased quickly inside flight chambers with small amounts of food (0.01–0.3 g) and then plateaued (Fig. 4B). Therefore, we did not include those points in the plateau (0.6 and 1 g) in the regression model describing flight initiation (Table 1). The number of progeny produced by females, regardless of their flight status, increased as quantity of food inside the flight chambers increased (Fig. 4C; Table 1).

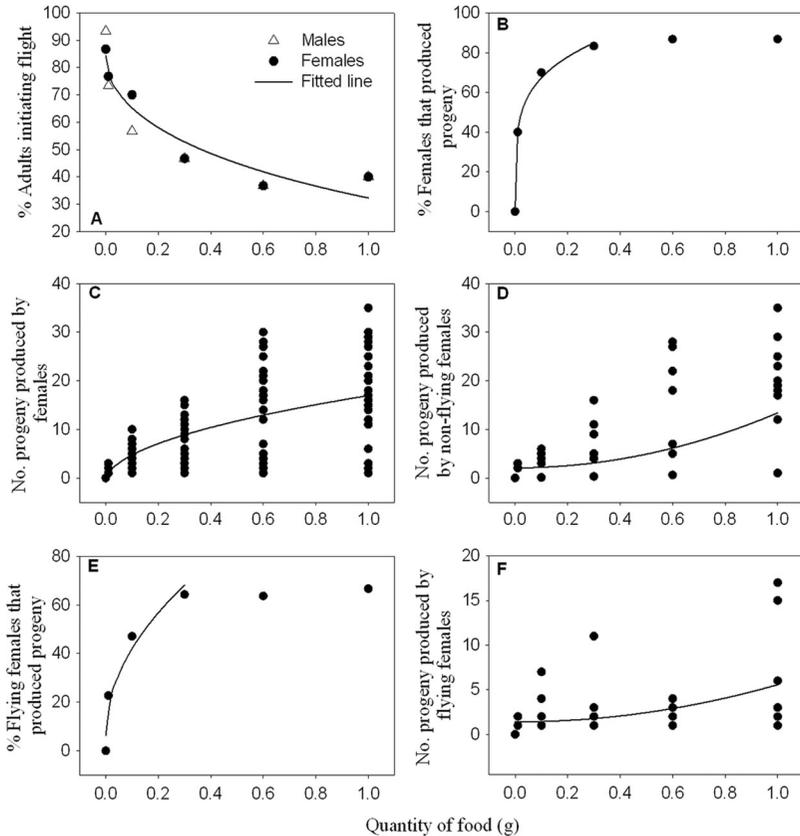


Fig. 4. Effect of quantity of food on flight initiation, percentage of females that produced progeny, and number of progeny produced by female *T. castaneum*. Parameters for the regression equations are in Table 1.

**Table 1.** Equations describing effects of quantity of food on rate of flight initiation, % females (all females and flying females) that produced progeny, and number of progeny produced by females (all females, and flying and nonflying females) inside flight chambers during a 3-d flight test

Process modeled <sup>a</sup>	a ± SE	b ± SE	R <sup>2b</sup>	Max. R <sup>2b</sup>
A. % adults initiating flight <sup>c</sup>	9.2 ± 0.4	-3.5 ± 0.7	0.90	
B. % females that produced progeny <sup>d</sup>	109 ± 4.7	0.211 ± 0.02	0.99	
C. No. progeny produced (all females) <sup>e</sup>	-0.9 ± 1.5	17.9 ± 2.2	0.35	0.37
D. No. progeny produced by nonflying females <sup>f</sup>	2.1 ± 1.2	11.3 ± 2.3	0.25	0.25
E. % flying females that produced progeny <sup>e</sup>	6.3 ± 5.5	113 ± 17	0.96	
F. No. progeny produced by flying females <sup>f</sup>	1.4 ± 0.7	4.2 ± 1.5	0.17	0.22

<sup>a</sup> Letters preceding processes modeled match letters on graphs in Fig. 4.

<sup>b</sup> R<sup>2</sup> is the amt of variation explained by the given equation; max R<sup>2</sup> indicates the max amt of variation that any equation fit to the data could explain, given the pure error in the data (Draper and Smith 1981).

<sup>c</sup> Equation is  $y^{0.5} = a + bx^{0.5}$ , where x = quantity of food (g) and y = % flight initiation.

<sup>d</sup> Equation is  $y = ax^b$ , where x = quantity of food (g) and y = % of females that produced progeny.

<sup>e</sup> Equation is  $y = a + bx^{0.5}$ , where x = quantity of food (g) and y = no. of progeny produced per female.

<sup>f</sup> Equation is  $y = a + bx^2$ , where x = quantity of food (g) and y = no. of progeny produced per female.

Females that did not fly were more likely to have produced progeny (96%) than females that did fly (36%). The number of progeny produced per female that did not fly increased with the amount of food inside the flight chamber (Fig. 4D; Table 1).

The percentage of females that produced progeny before flight increased rapidly in flight chambers containing 0.01, 0.01, and 0.3 g of diet ( $F = 42.6$ ;  $df = 1, 2$ ;  $P = 0.02$ ) and then plateaued (Fig. 4E). Therefore, we did not include those points in the plateau (0.6 and 1 g) in the regression model describing flight initiation (Table 1). The number of progeny produced by females that flew increased with the amount of food inside the flight chambers (Fig. 4 F; Table 1).

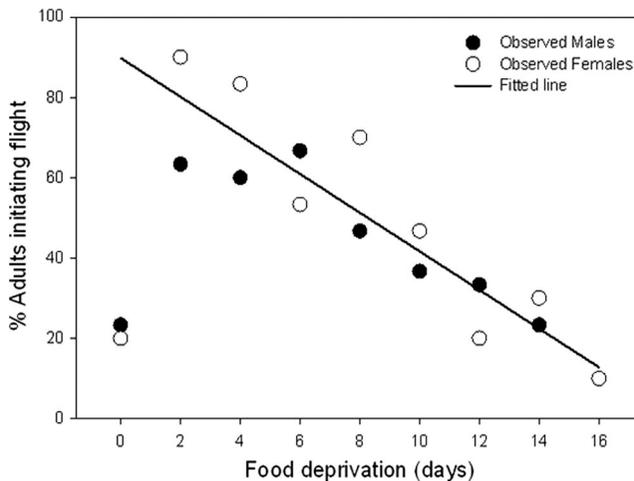
**Effect of Long-Term Food Deprivation on Flight Initiation.** No flight initiation occurred among adults deprived of food for more than 18 d so we did not use those data in the analyses. Flight initiation varied with duration of food deprivation ( $F = 6.9$ ;  $df = 8, 522$ ;  $P < 0.01$ ), but not with sex ( $F = 3.0$ ;  $df = 1, 522$ ;  $P = 0.08$ ) and the interaction also was not significant ( $F = 1.6$ ;

$df = 8, 522$ ;  $P = 0.14$ ). Therefore, we combined data for the sexes and described flight initiation as a function of food deprivation. Rate of flight initiation was higher for beetles deprived of food for short periods of time compared with flight initiation of beetles with food in the flight chamber. Flight initiation decreased with increasing time without food (Fig. 5).

The time of flight initiation did not vary with duration of food deprivation ( $F = 1.1$ ;  $df = 8, 234$ ;  $P = 0.37$ ) or sex ( $F = 0.7$ ;  $df = 1, 234$ ;  $P = 0.39$ ), and the interaction also was not significant ( $F = 0.6$ ;  $df = 8, 234$ ;  $P = 0.74$ ). The mean time of flight initiation for males and females combined was  $1.1 \pm 0.03$  d.

**Discussion**

Our results show that flight initiation of *T. castaneum* decreased as the beetles aged (Fig. 1). Our results support the hypothesis that the tendency for *T. castaneum* to migrate either by walking or flying persists throughout most of the adult life span (Ziegler 1976),



**Fig. 5.** Effect of food deprivation on flight initiation of male and female *T. castaneum*. The relationship between flight initiation (y) and duration of food deprivation (x) was described by the equation  $y = 89.76(5.5) - 4.814(0.5)x$ , ( $R^2 = 0.85$ ;  $n = 16$ ).

although beetles older than 165 d did not fly. Other stored-product beetles also have been shown to initiate flight more readily soon after adult emergence (e.g., *R. dominica*, Barrer et al. 1993, Aslam et al. 1994, Dowdy 1994); and *P. truncatus*, Fadamiro et al. 1996), but flight initiation of these other beetles only occurred in very young insects (<1-wk old or <2-wk-old, respectively) and was rare in older adults, unlike *T. castaneum* adults that were able to initiate flight even when 5 mo old. The relationship between age and flight initiation in *T. castaneum* is similar to that of many other insects that have the ability to fly throughout their adult life, for example, the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) (Schumacher et al. 1997), *Adelphocoris suturalis*, *A. fasciaticollis* Reuter, and the alfalfa plant bug, *A. lineolatus* (Goeze) (Heteroptera: Miridae) (Lu et al. 2009), and the ragweed beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) (Tanaka and Yamanaka 2009). The decline of flight initiation with age has been attributed to several causes such as degeneration of cell organelles, decline in glycogen reserves, genetically programmed expression of deleterious changes in respiratory enzymes, and the lack of protein synthesis after maturation (Sohal 1985). Progeny production also was dependent on age (Fig. 2); it began slowly in young females, but sharply increased in more mature females (57- to 120-d-old) before steadily decreasing in older females. In general, progeny production tended to parallel flight initiation, although more flight occurred in very young adults. Progeny production peaked in relatively young females that still showed high rates of flight initiation, and then both flight initiation and progeny production decreased with increasing age. This finding has pest management implications because, unlike other stored-product beetles, *T. castaneum* females are able to disperse and infest new commodities throughout most of their lives.

Mating status did not affect flight initiation of young (39- to 42-d-old) beetles supplied with food (Fig. 3). This finding does not conform with the oogenesis-flight syndrome where maximum migratory flight behavior is attained after the teneral period when the cuticle is hardened and immediately before the onset of reproduction (prereproductive stage), and flight usually does not occur in fully reproductive adults (Johnson 1969, Dingle 1985). Nonconformance with this syndrome also has been observed in a number of other species, for example, the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Gu and Danthanarayana 1990); the driedfruit beetle, *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae) (Blackmer and Phelan 1991); the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae) (Stewart and Gaylor 1991); the codling moth, *Cydia pomonella* (L.) (Schumacher et al. 1997); the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) (Sarvary et al. 2008); the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Han et al. 2008); and the ragweed beetle (Tanaka and Yamanaka

2009). It is likely in these species, and *T. castaneum*, that flight activity is just part of normal 'station keeping' (day-to-day movements associated with foraging) behaviors involved in exploiting patchy resources (Dingle 1996).

Flight initiation increased as quantity of food decreased (Fig. 4A). Changes in food quality and quantity have been shown to be important in influencing an adult's decision to initiate flight (Slansky 1982). Insects tend to respond to unfavorable conditions, such as food depletion, food deterioration, or adverse environmental conditions, by flying or walking away (Fadamiro et al. 1996, Perez-Mendoza et al. 1999, Dingle and Drake 2007). For example, mated females of the seed bug *Neocoryphus bicrucis* (Say) (Heteroptera: Lygaeidae) increase oviposition and fly less in the presence of good quality food, whereas oviposition is decreased and flight increases in the presence of poor quality food or when no food is present (Solbreck and Pehrson 1979). The convergent ladybeetle, *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae), showed a sharp decline in flight activity when in the presence of a high quality diet (Rankin and Rankin 1980). The larger grain borer, *P. truncatus*, maintained on whole maize showed less flight activity than when maintained on kibbled maize and maize flour which are poor diets (Fadamiro et al. 1996).

The presence of varying quantities of food inside the flight chambers impacted the number of progeny produced by *T. castaneum* females before flight (Fig. 4B), but not the timing of flight. Females produced more progeny in flight chambers with more food. Female red flour beetles have shown the ability to evaluate the size and the quality of a food patch and adjust the number of eggs that they lay in that patch in a way that they optimize production of adults from the patch (Campbell and Runnion 2003). The ability of *T. castaneum* to produce progeny before emigrating also was noted by Ziegler (1977). In his studies, emigrating females produced fewer progeny as the quality of food declined. The ability of *T. castaneum* to partition their reproduction between deteriorating local habitats and fresh habitats is very important for a species that is adapted to exploit widely distributed, sparsely populated, and very temporary habitats (Ziegler 1976). Therefore, for *T. castaneum*, the ability to continue flight with a fully developed batch of eggs would be advantageous for exploiting these types of habitats.

Food deprivation impacted flight initiation of both males and females, but flight initiation of *T. castaneum* was not completely inhibited until 18 d without food (Fig. 5). These results suggest that *T. castaneum* retain sufficient energy reserves to initiate flight even after long periods without food, although our methods did not allow us to determine how flight duration was impacted by these periods without food. The ability of this insect to survive long periods without food was noted by Daghish (2006), who observed that *T. castaneum* was more tolerant than *R. dominica* and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), to food deprivation, requiring up to 35 d of

starvation before progeny were not produced by females. Short periods of food deprivation have been shown to trigger flight initiation in other stored-product beetles, for example, periods of 24–72 h of starvation have increased the rate of flight initiation in *R. dominica* (Barrer et al. 1993, Perez-Mendoza et al. 1999) and *P. truncatus* (Fadamiro and Wyatt 1995).

Both sexes of *T. castaneum* exhibited great propensity for flight initiation, with no differences in flight tendencies between males and females in all the different experiments of this study. This is not surprising because flight seems to be part of normal foraging behaviors and a response to absence of food. In an earlier study, we found that rearing density, small differences in adult age (1- to 20-d-old), presence of food, short-term periods of food deprivation, and adult sex did not have any significant effect on flight initiation of this species.

Our results suggest that *T. castaneum* uses flight as a mechanism to disperse to new environments during almost any part of their life span and that this type of dispersion does not fit with the model of the so-called true migratory species that involves an “oogenesis-flight syndrome” (Johnson 1963). Adult females are able to produce some progeny before they initiate flight, suggesting that they are able to partition their own reproduction among several habitats, including their place of birth, while they are dispersing. Therefore, our results support Ziegler’s (1976) hypothesis that the emigration strategy of *T. castaneum* is well designed to colonize less stable habitats by responding to cues of these habitats in a more generalized way. This response would involve short-term physiological and behavioral adjustments to prevailing habitat conditions. It would permit a single adult female, through the proper timing of emigration, to partition optimally its reproductive effort between the original habitat and the habitat she is likely to colonize.

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