Activity of flour beetles (*Tribolium confusum)* in the presence of feces from rats infected with the rat tapeworm (*Hymenolepis diminuta*)

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Key words: *Hymenolepis diminuta*, *Tribolium confusum*, host behavior, parasite transmission, host finding

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ABSTRACT: We studied the attraction of flour beetles (*Tribolium confusum*) to feces from rats infected with the tapeworm *Hymenolepis diminuta.* Beetles were either fed or fasted prior to each trial. During trials beetles were tested singly or in groups and offered a choice (i) between natural baits made from fecal pellets from infected and uninfected rats, or (ii) between artificial baits made from feces of uninfected rats and differing only in the presence or absence of tapeworm tissue. Fasted beetles had a strong non-specific attraction to baits, while fed beetles tended to avoid baits. Fasted beetles also exhibited a greater ability to discriminate between control and infective baits, sometimes preferring infective baits but other times avoiding them. Experiments with artificial baits show that at least some of the signals that beetles respond to are of parasite origin. The results suggest that the foraging behavior of beetles in the presence of rat feces is more complex than previously thought, and includes the phenomena of attraction to, and avoidance of, feces from infected rats in situations whose parameters have yet to be identified conclusively.

Introduction

High parasite fecundity and chance encounters of eggs with invertebrate intermediate hosts may be the primary transmission strategy for cestodes with terrestrial life cycles (Mackiewicz 1988). Although Mackiewicz (1988) suggested that subtle mechanisms such as chemical attraction may increase the probability of eggs being eaten, he conceded that relatively little is known of the dynamics of predation on eggs. Evans et al. (1992) have since reported that flour beetles (*Tribolium confusum*), offered a choice between feces from rats infected with the rat tapeworm (*Hymenolepis diminuta*) and feces from uninfected rats, forage preferentially on the former. They suggested that this provides the first experimental evidence that a cestode can direct the feeding behavior of its intermediate host to favor transmission.

The experimental design of Evans et al. (1992) involved groups of fed or fasted beetles, placed in the centre of an arena. Control baits (feces from uninfected rats) and infective baits (feces from infected rats) were placed in semicircular bait zones on the periphery of a small arena. The number of beetles present in each zone was recorded at 20-min intervals. Evans et al. (1992) recognized that their experimental design could not provide information on the activities of individual beetles, and that social interactions within the groups of beetles may influence their response to baits. Pappas et al. (1995) used a different species of beetle (*Tenebrio molitor*) and a different experimental design: hosts of known age and sex, a larger arena, continuous monitoring of beetle locations, and control over the moisture content of the baits. Their analysis concentrated on the actual foraging activity of the beetles. Notwithstanding the differences in design and analysis, many results of Pappas et al. (1995) corroborated those of Evans et al. (1992): beetles preferred locations with a bait over locations without one, and fed and fasted females and fasted males preferred infective over control baits. Novel conclusions by Pappas et al. (1995) were that fed males avoided infective baits, that the preference of beetles for infective baits could not be explained by differences in moisture content, and that the response was not restricted to one species of beetle.

The objective of the present study was to further characterize the behavior of *T. confusum* in

the presence of feces from rats infected with *H. diminuta*. Like Pappas et al. (1995), our study manipulated the characteristics of the fecal material presented as baits, but whereas their study concentrated on sex differences in foraging behavior of beetles, ours emphasized analysis of the movements of beetles.

Materials and methods

Animals

We maintain *Hymenolepis diminuta* in male Sprague-Dawley rats, and *Tribolium confusum* in stock cultures with all-purpose flour kept in the dark at room temperature (20-24°C). The *H. diminuta* in our laboratory is a strain that originated from Rice University but has been supplemented with eggs of unknown origin from a biological supply house (J. C. Holmes, pers. comm.). Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Adult beetles (mixed sex and age) were removed from the stock cultures by placing a filter paper on the surface of the flour and collecting those that moved onto the paper. Some beetles ("fasted") were removed from food for 6 d prior to an experiment. (We note that *T. confusum* can survive > 2 weeks in the absence of food [Sokoloff 1974].) Others ("fed") were removed from the flour cultures immediately prior to the start of an experiment. For logistic reasons, the selection of beetles for experimentation from the stock cultures was not randomized, but once beetles were selected treatments were allocated randomly.

Four rats, 200-225 g at the start of the study, were the source of fecal material. Two rats were administered 20, 14-day-old cysticercoids each via gastric intubation following anesthesia with methoxyflurane. Twenty fecal pellets from these rats were examined for parasite eggs 21 days post-infection and all contained eggs. These were the source of infective pellets. Two other rats, not infected, were caged separately and were the source of control (non-infective) pellets.

Experimental Design

Four types of experiments were done, and each was repeated 2-3 times (Table 1). Experiments A and B had "continuous monitoring" (see below) of the location of individual beetles in the

presence of 0, 2, or 4 natural baits. Control arenas (no baits) were used to assess the movement patterns of beetles in the absence of bait. Two and four baits were used to assess the effects of the quantity of attractant material. Experiments C and D had "interval monitoring" (see below) on the locations of groups of 10 beetles in the presence of four natural baits (as in Evans et al. 1992) or four artificial baits. Each trial included fasted and fed beetles. Equal numbers of control and infective baits were used, placed equidistant in alternating sequence around the arena periphery.

Arenas were plastic petri dish bases (84 mm inside diameter) with a round paper insert glued to the inside as a textured substrate for the beetles. The insert had photocopied reference marks: either a 10 × 10 unit grid (8.4 × 8.4 mm squares), or zone markings as used by Evans et al. (1992). The inserts were prepared and glued on at least 24 h prior to each experiment to allow volatile compounds to dissipate. Baits were placed 1 grid unit from the periphery of the arenas immediately prior to the start of an experiment and beetles were introduced into the centre. The dish cover was placed on during observation. Each arena was used only once. Continuous, low lighting was used to minimize disturbance. Arenas were arranged in groups, 1 m beneath a 33-watt fluorescent light in an otherwise darkened room. A translucent corrugated plastic sheet was suspended 0.3 m beneath the light to act as a diffuser. Light intensity on the arenas, measured using a LI-188 Integrating Quantum/Radiometer/ Photometer with a LI-190s sensor (Lambda Industries, Lincoln NE) was 0.61 μmol photons $\cdot m^{-2} \cdot s^{-1}$. Within each group, individual arenas were oriented in various compass directions to minimize bias due to external environmental gradients. Beetles were counted into separate glass vials about 2 h prior to an experiment and placed under the test light source to acclimatize. At the start of an experiment they were poured through a glass funnel to introduce them into the centre of the arena.

Natural baits were prepared prior to the start of each experiment. The ends of fresh infective and control pellets were discarded and the remainder was transected into pieces about 5 mm long. Artificial baits were used to standardize the composition of the fecal material except for the presence or absence of tapeworm tissue. These baits were mechanically extruded cylinders of

fecal material prepared prior to each experiment. Each batch was prepared with 8.6 g fresh control pellets, soaked in 15 ml distilled water for 1 h, crushed using a mortar and pestle and divided into two portions. Into one portion (infective baits), 0.4 g of proglottids from the posterior ends of two *H. diminuta*, freshly recovered by necropsy, was ground in to rupture the proglottids and distribute parasite tissue and eggs throughout the mixture. The mixture was extruded through a 5-mm-diameter tube and cut into 5-mm-long segments. The remaining portion (control baits) received an additional period of grinding but no tapeworm tissue. A sample of control and infective baits was weighed fresh, and again after drying under ambient conditions for 5 d to assess similarity in size and moisture content. Factorial ANOVAs on fresh and dry weights of the artificial baits revealed no significant variation among batches, due to the presence of parasite tissue, or to interaction between the two ($P > 0.1$ in all cases). Mean fresh weight was 80 ± 11 (40) mg and mean dry weight was 26 ± 3 (40) mg (data are presented as $\bar{x}$ ± 1 SD (n) unless indicated otherwise). The average initial moisture content of these artificial baits (67%) was within the range (60-73%) determined similarly for freshly-deposited rat feces. Another sample of infective baits from each batch was examined for the number of tapeworm eggs. There was no difference between batches ($t_{[8]} = 1.49$, $P = 0.175$) and the overall mean was 1470 ± 566 (10) eggs per bait. The outer membranes of about one-half of the eggs were noticeably ruptured.

"Continuous monitoring" of locations was done on individual beetles in arenas with the grid reference markings. Data were recorded using a computer program that produced a facsimile of the arena grid on the monitor. After any change in location of a beetle (midpoint of body), a pointing device (mouse) was used to position the cursor on the corresponding location of the displayed grid. The arena number, new coordinates (resolution: ± 2.1 mm) and the time of observation were recorded automatically into a data file. New locations could be entered every 1-2 s during periods of beetle movement. During periods of beetle inactivity, coordinates of all beetles were re-entered at irregular intervals to provide confirmations on the time spent at those locations. A sample of these movement records is shown in Figure 1. Up to 12 arenas could be

monitored simultaneously if the beetle introductions were staggered at 5-min intervals (preliminary experiments had indicated that beetle activity was greatest during the first 5 min after introduction.) We believe that this system provided an accurate portrayal of beetle movement based on the following criteria: of 8,404 individual locations recorded during the study, the median distance moved was only 10 mm, and 95% of observations were recorded before the beetle had moved 1/2 the diameter of the test arena.

"Interval monitoring" of locations was done on groups of 10 beetles in arenas with the zone reference markings. Beetles were introduced into 24 arenas at 20 s intervals. Every 20 min, an observer noted the number of beetles in each bait zone (any part of body within the zone), proceeding from arena to arena at 20 s intervals.

Trials were 2 h in length. Arenas were observed from above and to the side, to reduce shadows. A sample of beetles from Experiment B was dissected immediately after the experiment to determine the sex ratio, and beetles from Experiments C and D were dissected after 2 weeks to determine the sex ratio and to count cysticercoids as confirmation that the beetles fed on the baits.

Data analysis

A computer program was written to take the sequence of location coordinates (Fig. 1) for continuously-monitored beetles in Experiments A and B and calculate the location, distance moved, mean velocity and position of beetles relative to baits during each time interval. Beetles within a radius of 0.5 grid sections of a bait (4.2 mm, about 1% of arena area) were considered to be at that bait. Some analyses were also done to determine whether beetle activities more distant from each bait were correlated with their appearances at a bait. Three sizes of hypothetical bait zones were created: small (1 grid unit radius around each bait, about 4% of arena area); medium (2 grid unit radius, about 12% of arena area); and large (any location nearer to one bait than to another, 50% of arena area when 2 baits were present and 25% when 4 baits were present). The drawn bait zones in Experiments C and D, similar to those used by Evans et al. (1992), each covered 8.5% of the arena.

Data from individual beetles (Experiments A and B) were analyzed in terms of the number of visits to a bait (each instance in which a beetle location changed from outside a bait zone to within one was considered to be a new visit) and the duration of each visit. Data from grouped beetles (Experiments C and D) were analyzed by comparing the proportion of beetles in bait zones to the area of the bait zone. Analyses included non-specific responses to baits (i.e. response to a bait whether control or infective) and discriminatory responses (i.e. choice of one type of bait over the other).

The arena, and not the individual beetles within it, was considered to be the lowest level of independent replication for statistical analysis because groups of beetles in an arena may not behave independently. The arcsin transformation was used on all proportion data, and the logarithmic transformation on other data when necessary to maintain homogeneity of variances for the analyses. Statistical procedures were based on Neter et al. (1985) and Sokal and Rohlf (1995). Analyses were conducted using SYSTAT statistical software (SPSS Inc. Staff, 1997).

Results

Observations on individual beetles (Experiments A and B)

Records of movements of individual beetles (e.g. Fig. 1) revealed a variety of qualitative patterns. The predominant component was movement around the periphery of the arena. Less common were direct movements from one point on the periphery to another across the central region of the arena, or erratic wandering in the central region of the arenas. When beetles encountered a bait they often circled it and occasionally climbed over it.

The mean velocity of continuously monitored beetles was affected significantly only by time after introduction (factorial ANOVA, repeated measures over time: $F_{[10,660]} = 29.4$, $P < 0.001$). The mean velocity of beetles (Fig. 2) was high initially and then declined rapidly during the first 10 min after introduction into an arena. There was no difference ($P > 0.15$ in all cases) between fed or fasted beetles or among beetles in the presence of 0, 2, or 4 baits, and there was no interaction effect.

Non-specific responses to baits were determined from the number and duration of visits to a

bait during the 2-h observation period. The number of visits was not affected (factorial ANOVA: $P > 0.25$ in all cases) by the number of baits present or beetle satiation, and there was no interaction effect. Beetles made 5.0 ± 4.8 (54) visits to a bait per 2 hr trial. However, 27/27 fasted beetles visited at least one bait, whereas fewer (21/27) fed beetles visited at least one bait (Fisher's exact test, $P = 0.023$). The mean duration of visits to a bait was calculated for each continuously-monitored beetle. These 54 beetles made a total of 271 individuals visits to baits, which were highly variable in length (514 ± 939 s, with a median of 94 s.) A mean visit length was calculated for each beetle. The mean visit length of each beetle was not affected (factorial ANOVA: $P > 0.15$ in all cases) by satiation or the number of baits present, and there was no interaction effect. These means were also highly variable (2580 ± 2309 s (54), with a median of 2387 s.)

Discriminatory responses to baits were tested by two methods. In the first method, the number and length of visits to baits was compared using a paired *t*-test (Table 2) to reduce the effects of the high variability among beetles described previously. Fasted beetles made significantly fewer visits to infective baits than to control baits, when either 2 or 4 baits were used. The number of visits by fed beetles did not differ significantly. The duration of visits to control and infective baits did not differ significantly for fed or fasted beetles in the presence of 2 or 4 baits (Table 2). In the second method, confidence limits for the mean time spent by each beetle at control and infective baits were calculated (Table 3). These were compared to the null hypothesis that beetles not responding to baits would spend similar time in all parts of the test arena. Beetles spent the same or more time at control baits than expected by chance, but significantly less time at infective baits than expected by chance in 3 of 4 comparisons (Table 3).

Observations on beetles at baits were highly correlated with the observations that would have been made if small- or medium-sized bait zones had been used (Table 4). After partitioning the data by number of baits and beetle status, correlations with a small bait zone were significant in all but two cases, and correlations with a medium-sized bait zone were significant in more than one-half of cases. The large bait zones, however, produced poor correlations.

Observations on grouped beetles (Experiments C and D)

Non-specific responses to bait were tested by comparing the number of beetles in bait vs. non-bait zones (Table 5). Feeding status of the beetles and time after introduction had a highly significant effect in both experiments, but there was no significant variation among trials. Based on these results, trials were pooled and tests of preference for baits used data segregated by time and satiation for both experiments.

The null hypothesis, that beetles distributed at random would be located in bait zones in proportion to the size of the bait zone, was rejected for at least some time intervals in all experiments (Fig. 3). There was a general tendency for the increased occurrence of beetles in bait regions over time. Fed beetles presented with natural baits were located in a bait region less often than expected for the first 60 min, but thereafter were distributed at random (Fig. 3a). Fasted beetles were distributed initially at random, but after 20 min they consistently occurred in a bait region more often than expected (Fig. 3a). Fed beetles presented with artificial baits were located in a bait region less often than expected throughout the experiment (Fig. 3b). Fasted beetles were initially located in an artificial bait region less often than expected, but after 40 minutes they occurred there more often than expected (Fig. 3b). More fasted beetles than fed beetles were observed in bait zones at all times (Fig. 3a,b).

Discriminatory responses to bait were tested by comparing the number of beetles in infective-bait zones vs. control-bait zones (Table 6). Feeding status of the beetles and time after introduction had a highly significant effect only in Experiment D, and there was no significant variation across time or among trials. Based on these results, we chose to pool trials, and subsequent tests of preference for infected bait regions used data segregated only by time for Experiment C, and by time and feeding status for Experiment D.

The null hypothesis that beetles in a bait zone show equal preference for infective vs. non-infective baits was rejected for only a few time intervals in Experiments C and D (Fig. 4). Beetles presented with four natural baits preferred control baits during two intervals but had no preference during the other four (Fig. 4a). Fed beetles presented with artificial baits preferred

control baits during two intervals but had no preference during the other four (Fig. 4b). Fasted beetles preferred infective artificial baits during one time interval but had no preference during the other five (Fig. 4b).

Sex ratio and infections of beetles

The sex ratio of beetles from Experiment B and D did not differ from 1:1 (G-tests: Experiment B, 12/24 male, $G_{[1]} = 0$, $P = 1.0$; Experiment D: 14/25 male, $G_{[1]} = 0.35$, $P = 0.552$), but the sex ratio from Experiment C was female-biased (10/50 male, $G_{[1]} = 19.1$, $P < 0.001$). Beetles exposed to natural infective baits acquired an infection. Examination of 50 beetles from Experiment C, 3 weeks post-infection resulted in recovery of 1-13 cysticercoids from 11 beetles (22% infected; 1.1 ± 2.9 cysticercoids/beetle). No cysticercoids were found in 25 beetles from Experiment D examined 3 weeks after exposure to artificial infective baits.

Discussion

This study examined a number of behaviors of *Tribolium confusum* that relate to its likelihood of encountering feces of rats infected with *Hymenolepis diminuta*, and acquiring an infection from them. Results on non-specific responses corroborated previous conclusions (Evans et al. 1992; Pappas et al. 1995). Beetles preferred locations in the vicinity of rat feces. There was a differential response between fed and fasted beetles. Beetles foraged on infective baits and acquired infections. Beetles gradually accumulated around baits as each experiment progressed. On the central question of discriminatory responses between infective and control feces, our results were somewhat contradictory. While beetles discriminated between the two, they often preferred control over infective baits. Moreover, in some experiments they actually avoided infective baits.

The first evidence that the preference of beetles for infective feces is not absolute was provided by Pappas et al. (1995), who found that male *Tenebrio molitor* avoid infective feces. The variety of situations in which we observed avoidance demonstrates convincingly that preference for infective feces cannot be regarded as a general phenomenon even for the *T. confusum — H. diminuta* relationship. Some instances of avoidance involved individual beetles;

others, grouped beetles. Some involved fasted beetles; some, fed beetles; yet others applied to all beetles. Avoidance was observed with two or four natural baits and with artificial baits. The specific factors that cause preference or avoidance of infective baits in a given situation remain unclear. Several factors have been hypothesized (Evans et al. 1992; Pappas et al. 1995) to play a role. These fall under two general categories: the presence of specific attractants, and the modification of foraging behavior by social situation, hunger, age or sex of the beetle.

The presence of an attractant substance in feces of infected rats was postulated by Evans et al. (1992), who suggested that it could be of parasite origin or produced by the host in response to infection. Pappas et al. (1995) concluded that the attractant substance was not volatile, but our observation that fasted beetles made fewer visits to infective baits suggests that there may be some substance that beetles can detect at a distance. Pappas et al. (1995) observed casually that *T. molitor* seems to prefer moister baits, but they found that infective baits were still preferred over controls after desiccating and then rehydrating them to a similar water content. Sokoloff (1974) noted that most members of the genus *Tribolium* avoid moist food items in preference for dry ones. Our results with artificial baits also suggest that when moisture content is controlled, *T. confusum* has a weak ability to discriminate among infective and control feces. Since the fecal matter for the artificial baits was from uninfected rats, indirect attractants resulting from a modification of host physiology by tapeworm infection should not have been present. The maceration of proglottids during preparation of baits may have altered the concentration of parasite-derived substances, but should not have introduced new substances into the baits because disrupted proglottids occur naturally. Our evaluation of the results of Evans et al. (1992), Pappas et al. (1995) and the present study suggests that beetles can discriminate among infective and control fecal pellets, that water content (the only substance of host origin that has yet been tested) is not the basis for this, and that some substance of parasite origin can be detected by beetles.

Evans et al. (1992) suggested that aggression or the existence of a feeding hierarchy would explain why some fasted beetles apparently fed on control baits even though a majority preferred

infective baits. Fasted as well as fed beetles monitored individually in our study, and thus not in a feeding hierarchy, not only visited control baits but did so preferentially in many cases. Many beetle behaviors vary with sex, age, and density (Sokoloff 1974). We obtained substantially similar results for beetles assayed individually or in groups of 10, indicating that the behavior of beetles towards baits in the test arenas was independent of beetle density. This suggests that the preference of beetles for infective feces in other studies (Evans et al. 1992; Pappas et al. 1995) was not necessarily a function of the social situations created in their test arenas.

Our study corroborates previous conclusions (Evans et al. 1992; Pappas et al. 1995) that the beetle's state of satiety modifies their response to fecal pellets and their ability to discriminate between infective and control pellets. *Tribolium castaneum* normally exhibits a "dry reaction", avoiding high humidity locations, but they are attracted to high humidity after fasting (Sokoloff 1974). Fasted beetles may have exhibited greater attraction than fed beetles to fecal pellets simply due to their water content. The accumulation of beetles around baits as experiments progressed may simply reflect the normal "dry reaction" as the pellets dried and beetles were no longer repelled. Our data also refute another hypothesis proposed by Evans et al. (1992): that the preference of fed beetles for infective baits results from brief but frequent visits to infective baits. We found no difference among bait types in number or length of visits by fed beetles.

The modifying effects of host sex are not as clear as the effect of host satiety. Fed male *T. molitor* avoid infective pellets whereas fasted males or fed or fasted females are attracted (Pappas et al. 1995). Although our study was not designed to study the effect of host sex, we observed one of the strongest avoidances of infective baits by fasted beetles in Experiment B, which had a female-biased sex ratio. If host sex is a contributing factor, its effects may depend on complex interactions with other factors not tested to date.

The results from Evans et al. (1992), Pappas et al. (1995) and the present study suggest that the three sets of experimental designs had enough in common to identify similar non-specific responses to baits, with the disparate results primarily concerning discriminatory responses. This suggests that the phenomenon of a preference for infective baits is determined by relatively

subtle factors. Various combinations of rat, beetle and parasite material were used in these studies. Wistar rats were used by Evans et al. (1992), and Sprague-Dawley rats by Pappas et al. (1995) and us. Only Pappas et al. (1995) used a characterized parasite strain. These variations may contribute to differences in results between laboratories. Strains of *H. diminuta* from various sources differ in properties such as egg morphology (Pappas and Leiby 1986). Susceptibility to infection with *H. diminuta* (Yan and Norman 1995) and behavior following infection (Yan, Stevens and Schall 1994) varies among strains of *T. confusum*. Metabolic end products differ between and within combinations of host and parasite strains (Bennet et al. 1990).

There were also differences in the arenas used in the three studies. The arenas used by Pappas et al. (1995) were larger (as was the species of beetle being studied) but their physical composition was not described. The arenas used in our study differed from those used by Evans et al. (1992) in two ways. First, our arenas used paper inserts rather than letting the beetles crawl directly on the plastic. We felt there was a benefit to providing a three-dimensional substrate that more closely resembled the flour on which the beetles normally moved, and also enabled beetles to right themselves more easily when they tipped over. These inserts may release odors that could mask the substance contained in infective baits. However, these factors were likely dissipated in the 24-hr delay between construction and use of arenas, because fasted beetles were able to discriminate between baits even when only one bait of each type was present. Second, our arenas were covered with the petri dish lids and not disturbed during the observation period. Evans et al. (1992) covered the arenas with paper towelling and removed it at 20-min intervals to record observations. This disturbance may have affected overall levels of beetle activity although clearly it did not disrupt the ability of beetles to discriminate between baits in that study.

We introduced some novel methods to the study of beetle response to rat feces. Recordings of individual beetle locations provided a detailed account of beetle presence at baits. This enabled us to detect discriminatory responses to baits, but was laborious to conduct and analyze. This method also permitted us to conduct correlation analyses which confirmed that the much

simpler procedure of recording of beetle presence in bait zones, a method devised by Evans et al. (1992) and used in our Experiments C and D, is a good predictor of bait choice by beetles. The use of artificial baits is a promising method to control and manipulate the source of potential attractant materials.

Our study suggests that *T. confusum* are highly heterogeneous in behavior, both among individuals and by the same individual over time. Although some of this heterogeneity was likely due to the mixed age and sex of beetles used in the trials, we were nevertheless able to demonstrate statistically that *T. confusum* are not only attracted to rat feces and able to acquire an infection from them, but can discriminate subtle differences in the composition of baits presented to them. We confirmed that beetles are not always attracted to infective host feces and sometimes avoid them. Our novel methods have helped to test some existing hypotheses, and our results suggest strongly that this phenomenon is multifactorial in origin.

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Table 1. Experimental designs and sample sizes.

			Beetles/arena		No. arenas	
Exp't	Baits [a]	Trial	No.	Status [b]	Control	With bait
A	1C, 1I; Natural	1	1	Fed	2	4
			1	Fasted	2	4
		2	1	Fed	1	5
			1	Fasted	1	5
B	2 C, 2 I; Natural	1	1	Fed	4	8
			1	Fasted	4	8
		2	1	Fed	1	5
			1	Fasted	1	5
		3	1	Fed	1	5
			1	Fasted	1	5
C	2 C, 2 I; Natural	1	10	Fed	0	12
			10	Fasted	0	12
		2	10	Fed	0	12
			10	Fasted	0	12
D	2 C, 2 I; Artificial	1	10	Fed	0	13
			10	Fasted	0	11
		2	10	Fed	0	13
			10	Fasted	0	11

[a] C = control bait; I = infective bait

[b] Fed = removed from food immediately prior to experiment; Fasted = without food for 6 days prior to experiment.

Table 2. Number and duration of visits to control and infective baits (Experiments A and B).

			Bait type		Paired *t*-test [a]	
	Baits	Status [b]	Control [c]	Infective	df	*P*
Number						
($\log_{n+1}$)	2	Fed	0.38 ± 0.38 (9)	0.23 ± 0.36 (9)	8	0.397
		Fasted	0.51 ± 0.29 (9)	0.17 ± 0.33 (9)	8	0.045
	4	Fed	0.43 ± 0.35 (18)	0.46 ± 0.34 (18)	17	0.577
		Fasted	0.58 ± 0.31 (18)	0.31 ± 0.34 (18)	17	0.003
Duration						
($\log_{s+1}$)	2	Fed	2.48 ± 0.47 (6)	1.86 ± 0.77 (3)	2	0.557
		Fasted	2.22 ± 1.13 (9)	1.98 ± 1.06 (3)	2	0.453
	4	Fed	2.27 ± 0.71 (12)	2.56 ± 0.71 (13)	9	0.474
		Fasted	2.74 ± 0.54 (16)	2.07 ± 0.87 (10)	7	0.446

[a] Pairs are data from control and infective baits within same arena.

[b] Fed = removed from food immediately prior to experiment; Fasted = without food for 6 days prior to experiment.

[c] $\bar{x}$ ± SD (*n*) for all observations; sample size used in paired *t*-tests on visit length are smaller because not all beetles visited both types of baits.

Table 3. Time spent by beetles at a bait and the expected time based on the proportion of arena comprising the bait zone (Experiments A and B).

Baits	Status [a]	Expected (s)	Bait type	
			Control	Infective
2	Fed	72	4 - 1244 [b]	0 - 64 ⇓
	Fasted	72	30 - 3684	0 - 56 ⇓
4	Fed	144	13 - 374	28 - 916
	Fasted	144	205 - 2897 ⇑	4 - 124 ⇓

[a] Fed = removed from food immediately prior to experiment; Fasted = without food for 6 days prior to experiment.

[b] 95% Confidence limits for mean s recorded at each type of bait. Confidence limits were calculated on transformed ($\log_{s+1}$) data and back-transformed for presentation. Arrows indicate significantly greater (⇑) or lesser (⇓) time spent than expected based on area of bait zone.

Table 4. Correlations between observations on beetles at a bait, with those same observations had bait zones of various size been used (Experiments A and B).

				Size of bait zone [a]		
	Baits	Status [b]	*n*	Small	Medium	Large
Total no. visits	2	Fed	9	0.60 [c]	0.45	0.00
		Fasted	9	0.85*	0.60	-0.26
	4	Fed	18	0.90***	0.89***	0.37
		Fasted	18	0.74**	0.56	0.25
Time in infective bait zone	2	Fed	9	1.00***	0.79	0.60
		Fasted	9	0.98**	0.97***	0.16
	4	Fed	18	0.98***	0.93***	0.67*
		Fasted	18	0.78***	0.59	0.39
Time in control bait zone	2	Fed	9	0.98***	0.66	0.43
		Fasted	9	0.99***	0.95***	0.60
	4	Fed	18	0.73**	0.64*	0.10
		Fasted	18	0.97***	0.88**	0.69**
Proportion of time in infective bait zone	2	Fed	9	1.00***	0.99***	0.99***
		Fasted	9	0.58	0.59	0.00
	4	Fed	18	0.99***	0.88***	0.67*
		Fasted	18	0.64*	0.75**	0.55

[a] Zone around each bait as percent of arena area: small, 4%; medium, 12%; large, 50% when 2 baits present and 25% when 4 baits present.

[b] Fed = removed from food immediately prior to experiment; Fasted = without food for 6 days prior to experiment.

[c] Pearson correlation coefficients and Bonferroni-corrected probabilities: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Table 5. Summary of repeated-measure ANOVAs on preference of beetles for baits (Experiments C and D).

	Exp't C		Exp't D	
Source	df	*P*	df	*P*
Between arenas				
Status [a]	1	< 0.001	1	<0.001
Trial [b]	1	0.810	1	0.052
Error	45		43	
Within arenas				
Time [c]	5	< 0.001	5	<0.001
Time × Status	5	0.228	5	<0.001
Time × Trial	5	0.264	5	0.124
Error	225		215	

Note: Preference was measured as the proportion of beetles in an arena at a bait vs. not at a bait.

[a] Fed or fasted beetles.

[b] 2 or 3 repetitions of each experiment.

[c] 6 consecutive 20-min time intervals.

Table 6. Summary of repeated-measure ANOVAs on preference of beetles for infective baits (Experiments C and D).

	Exp't C		Exp't D	
Source	df	*P*	df	*P*
Between arenas				
Status [a]	1	0.648	1	<0.001
Trial [b]	1	0.552	1	0.687
Error	34		22	
Within arenas				
Time [c]	5	0.477	5	0.138
Time × Status	5	0.407	5	0.098
Time × Trial	5	0.365	5	0.343
Error	170		110	

Note: Preference was measured as the proportion of beetles in an arena at an infective vs. control bait.

[a] Fed or fasted beetles.

[b] 2 or 3 repetitions of each experiment.

[c] 6 consecutive 20-min time intervals.

FIGURE CAPTIONS

Figure 1. Example of beetle locations recorded in Experiment B during continuous monitoring over a 2-h period in the presence of two infective (shaded rectangle) and two control (open rectangle) baits. Circles represent the locations at which recordings were made, and lines connect sequential records.

Figure 2. Mean velocity of beetles during various time intervals after introduction into test arenas, $cm \cdot min^{-1}$ (pooled data from Experiments A-B, 0-4 baits present, $\bar{x} \pm 95\%$ confidence limits, $n = 72$ beetles).

Figure 3. Proportion of beetles in any bait region, at 20-minute time intervals after introduction into arenas. (a) Four natural baits, Experiment C. (b) Four artificial baits, Experiment D. Data are represented as $\bar{x} \pm 95\%$ confidence limits. ○: fasted beetles; ●: fed beetles. Horizontal line represents expected proportions based on area of bait region relative to arena.

Figure 4. Proportion of beetles in any bait region that were nearest to an infective bait, at 20-minute time intervals after introduction into arenas. (a) Four natural baits, Experiment C. (b) Four artificial baits, Experiment D. Data are represented as $\bar{x} \pm 95\%$ confidence limits. ○: fasted beetles; ●: fed beetles; ■: pooled data. Horizontal line represents expected proportions based on equal occurrence in control-bait and infective-bait regions.

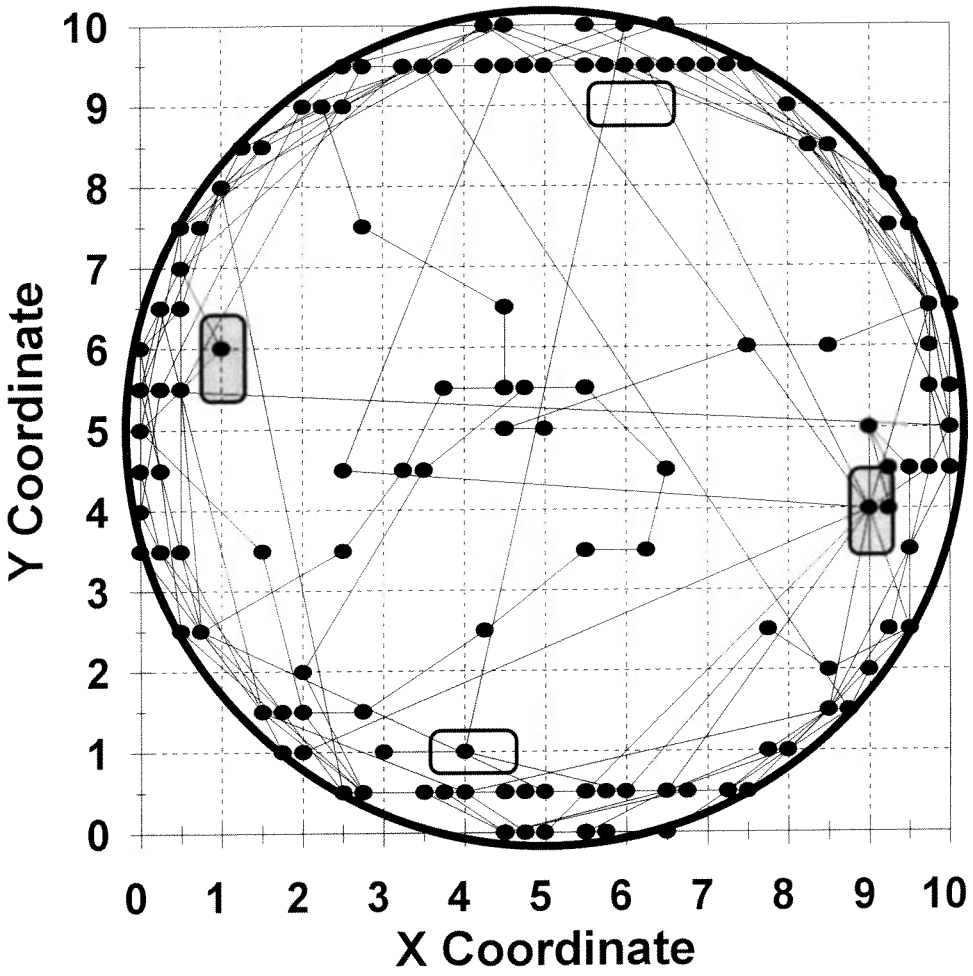

Figure 1: TOP ⇑
Shostak & Smyth Y1357

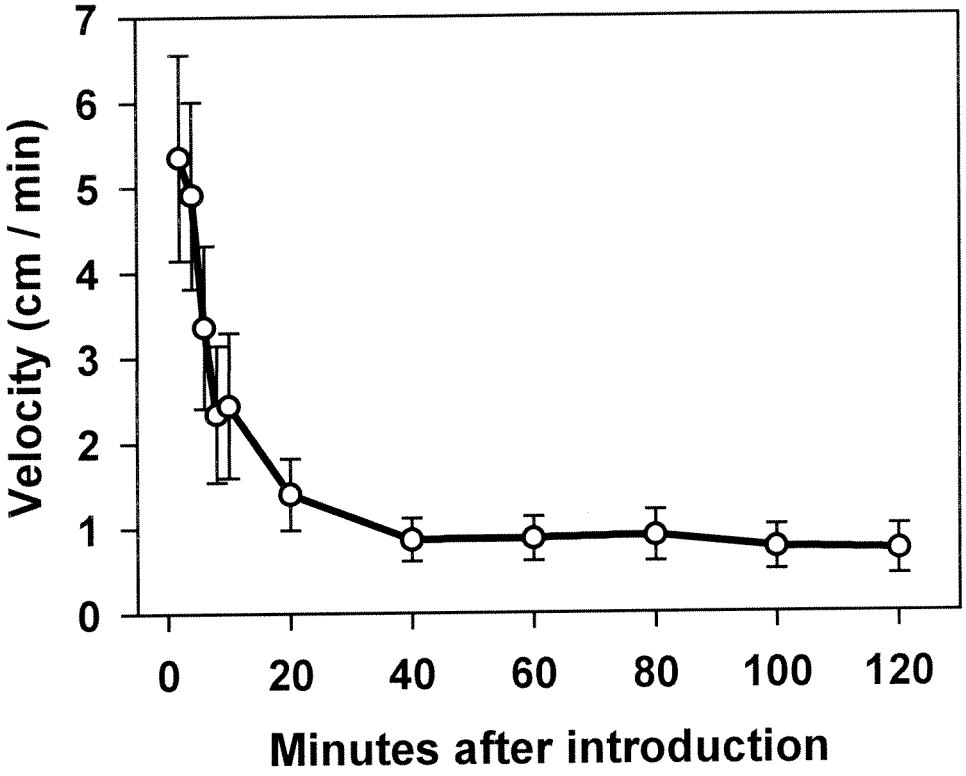

Figure 2: TOP ⇑
Shostak & Smyth Y1357

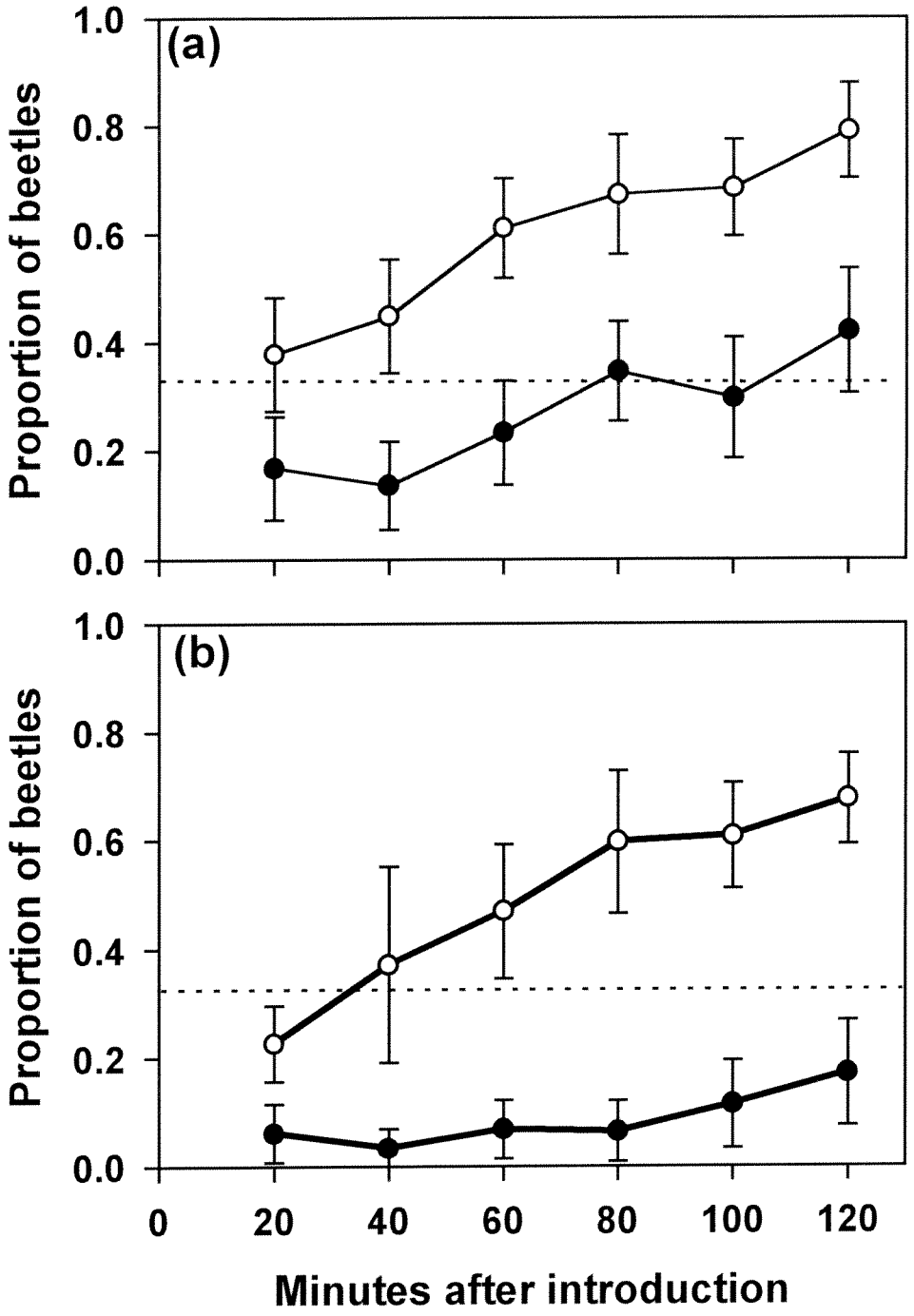

Figure 3: TOP ⇑
Shostak & Smyth Y1357

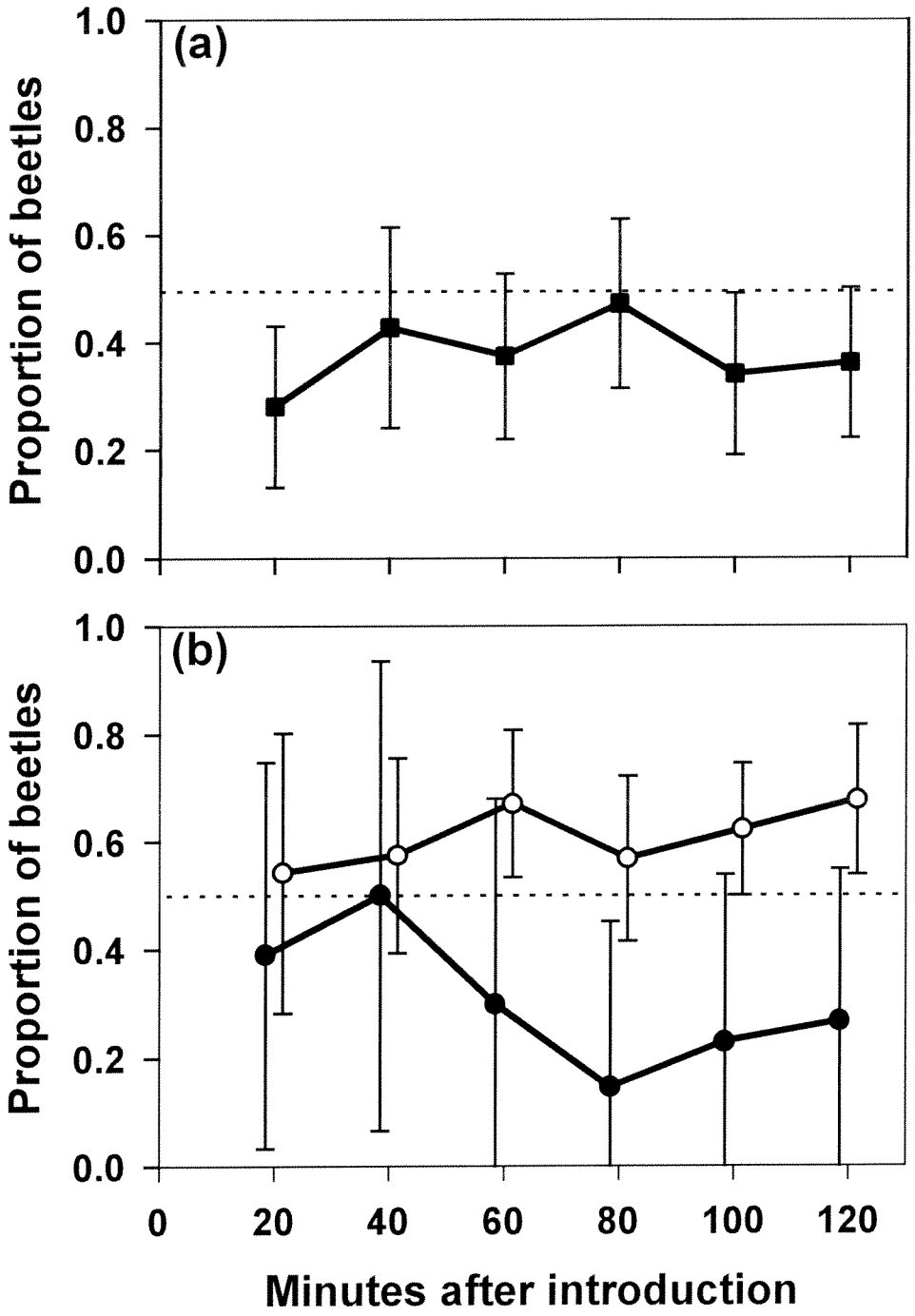

Figure 4: TOP ⇑
Shostak & Smyth Y1357