

BEHAVIORAL CHANGES IN *TRIBOLIUM* BEETLES INFECTED
WITH A TAPEWORM: VARIATION IN EFFECTS BETWEEN
BEETLE SPECIES AND AMONG GENETIC STRAINS

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Abstract.—We report behavioral alterations induced by tapeworm (*Hymenolepis diminuta*) infection in several genetic strains of two species of flour beetles, *Tribolium*, under two infection levels in a laboratory environment. Under a high level of infection, *Tribolium castaneum* (strain cSM) shows decreased emigration and surface-seeking behavior and increased cannibalism. In contrast *Tribolium confusum* (strain b+) shows increased emigration and surface-seeking behavior, and cannibalism is not significantly changed. Under a low level of infection, emigration is not affected in any of the 11 genetic strains examined. Prevalence of parasitism is positively associated with emigration rate. Different genetic strains of *T. confusum* exhibited different susceptibility to tapeworm infection, suggesting intraspecific genetic variation in susceptibility to infection. This genetic basis may reflect past differences in selection resulting from different infection histories and suggests susceptibility to infection could evolve as a result of future selection. This variation in changes in host behavior seen among genetic strains also suggests that caution is needed before generalizing about expected changes in host behavior induced by a particular parasite and before concluding that such changes represent an adaptive manipulation of host behavior by a parasite species.

Parasites can alter the behavior of infected hosts in striking ways (reviews in Holmes and Bethel 1972; Moore 1984a; Molyneux and Jefferies 1986; Moore and Gotelli 1990). In some cases, such as parasites with complex life cycles that are transmitted through the food chain, alteration in the host's behavior might influence transmission rates (Holmes and Bethel 1972; Moore 1983a, 1984b; Quinn et al. 1987; Gotelli and Moore 1992). For example, amphipods (*Gammarus lacustris*) infected with acanthocephalan cystacanths of *Polymorphus minutus* were more often found at the top of the water column (Hindsbo 1972). Isopods (*Armadillidium vulgare*) infected with the acanthocephalan parasite, *Plagiorhynchus cylindraceus*, were more frequently found in light-colored substrates and in unsheltered areas than were noninfected individuals (Moore 1983b), and *Tenebrio* beetles infected with the rat tapeworm *Hymenolepis diminuta* exhibited decreased activity and photophobic behavior (Hurd and Fogo 1991). These behavioral changes could leave the hosts more susceptible to predation by the definitive host. It has been proposed that such altered host behavior is an example of

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adaptive manipulation by the parasite resulting from natural selection favoring any trait in the parasite that increases transmission success (Moore 1984a; but see Moore and Gotelli 1990). This hypothesis has been challenged, though, by some authors (Smith Trail 1980; Dawkins 1982; Minchella 1985; Hart 1990) who argue that changes in host behavior may be an expression of traits that originally or currently benefit hosts, in altering preferred temperatures, energy, and/or diet, or are merely generalized pathology from damage caused by the parasite (see, e.g., Boorstein and Ewald 1987; Smith and Kramer 1987; Hart 1990). Indeed, unequivocal demonstration that altered host behaviors represent adaptive manipulation by the parasite is difficult to achieve (Moore and Gotelli 1990).

Even if alteration of host behavior during infection is not an adaptation on the part of the parasite or host, such behavioral changes could have important ecological consequences for both (Dobson 1988). For example, behavioral changes that fortuitously alter the parasite's transmission success would still have an impact on parasite-host dynamics, and yet other behavioral changes could alter the population biology or interspecific interactions of the host, even in the absence of effects on parasite transmission.

In this article we explore these issues with a study of the consequences of infection of the rat tapeworm, *H. diminuta*, on host behavior in several strains of each of two species of flour beetles, *Tribolium confusum* and *Tribolium castaneum*. Both beetles are cosmopolitan and are conveniently cultured in the laboratory under uniform climatic and nutritional regimes. The adult parasite lives in nature in the lumen of the small intestine of rats, where the eggs are produced and passed out with the host's feces. Further development takes place when the eggs are ingested by the proper intermediate host, often grain-infesting insects such as *Tribolium* and *Tenebrio* beetles. This life cycle can be readily maintained under laboratory conditions; prevalence and intensity of parasitism are easily determined by dissection. Prevalence of parasitism is defined as the proportion of host individuals in a sample infected with the parasite. Intensity of parasitism is defined as the number of parasites in each host individual exposed to the parasite eggs (Margolis et al. 1982). Because the infection can be controlled and monitored and the ecology and genetics of the beetle's behavior are well understood, the association between *H. diminuta* and *Tribolium* is an ideal system to study parasite-induced altered behavior.

We studied the effect of tapeworm infection on three behaviors (surface-seeking behavior, emigration rate, and cannibalism) and one life-history trait (fecundity) of two host species of flour beetles (*T. castaneum* and *T. confusum*). We chose these traits because they are interrelated, are known to show genetically based variation among species and strains of *Tribolium*, and are likely to influence parasite transmission and the population and community ecology of the beetles. Different strains and species of flour beetles are known to differ in their emigration rate and their tendency to be on the surface of the flour medium (Wu 1981); this seems a behavior likely to affect parasite transmission because beetles on the flour surface may be more prone to predation by rats in nature. The rate of cannibalism of eggs by adult beetles also varies among strains and species (Park et al. 1964; Stevens 1989). *Tribolium castaneum* is generally more cannibal-

istic than is *T. confusum* (Park 1948; Park et al. 1965) and as a result usually dominates in interspecific competition (Park 1948, 1954, 1957), but it is also more likely to become infected with parasites transmitted through feeding, such as the protozoans *Adelina tribolli* (Park 1948) and *Nosema* spp. (G. Yan, personal observation) and the cestode *H. diminuta* (below). Fecundity is one of the most important life-history traits of flour beetles, and it has been shown that selection in emigration rate on *T. confusum* and *T. castaneum* could change many life-history traits (Wu 1981). This suggested to us that parasitism could influence host community structure through the impact of parasitism on host emigration behavior and fecundity.

One strain from each beetle species was examined for all traits at high prevalence and high intensity of parasitism. In addition, emigration rate was measured for several strains of each species under low prevalence and low intensity of parasitism. This allowed us to compare the effect of intensity of parasitism on host behavior, to determine the generality of our results, and to examine the among-strain genetic variation for the altered behaviors. Early studies of altered host behavior suggested unvarying stereotypical behavioral responses. More detailed investigations revealed considerable variability among individual hosts in their responses to parasitism. This variability may indicate the presence of underlying genetic variability for behavioral responses to parasitism. Genetic variability in host responses has not been well studied but is an important element in our understanding of the evolution of altered host behavior. The strains used here were isolated from geographically distinct populations and have been isolated for years in laboratory culture. Differences among strains in their response to parasitism could reflect either past differences in selection experienced by these populations or the potential for future selection. Because the same parasites were used to infect the beetles, among-strain differences in host behavior are due solely to genetic differences among hosts.

MATERIAL AND METHODS

Unless otherwise stated, beetles were raised in 28-mm-diameter \times 95-mm-shell vials containing 8 g standard medium (95% by weight fine-sifted whole wheat flour and 5% dried powdered brewer's yeast). The vials were maintained in a dark incubator regulated at 29°C and 70% relative humidity.

Hymenolepis diminuta has long been used as a model for a variety of physiological and pathological studies of cestodes (see, e.g., Arai 1980; Thompson et al. 1986; Hurd and Weaver 1987; Wages and Roberts 1990; Dixon and Arai 1991). *Hymenolepis diminuta* eggs were obtained as infected rat feces from Carolina Biological Supply (Burlington, N.C.). When *Tribolium* ingests tapeworm eggs, development of the cysticeroid larvae takes place in the hemocoel of the insect and the mature cysticeroids can be dissected out of the beetles after about 2 wk (Cheng 1973; Keymer 1980). Cysticeroids are infectious only to a mammalian host and are not transmitted horizontally among beetles.

Host Behavioral Alterations under High Infection Levels

One genetic strain of each of two species of flour beetle was used to examine the effects of a high level of infection on host behavior. We studied the cSM strain of *Tribolium castaneum* and the b+ strain of *Tribolium confusum*. The strain b+ has been maintained in laboratory culture since the 1930s (Stevens 1989), and cSM originated by combining several laboratory strains in the 1970s (Wade 1977).

Beetle infection.—Twenty adult flour beetles 7 d posteclosion were starved for 1 wk. After starvation the beetles were exposed to approximately 0.5 g of infected rat feces mixed in 0.3 mL distilled water on a 2 × 6.5-mm strip of filter paper. All the beetles were exposed to the same amount of infected rat feces. Control beetles were also starved and given distilled water on filter paper for 24 h. After 24 h the filter paper was removed and 8 g of flour medium was added to each vial. About 800 beetles of each species and each sex were simultaneously infected in this method (800 beetles per strain and sex ÷ 20 beetles per vial = 40 vials of beetles per species and sex). Of these about 19 vials of beetles were used in the emigration experiment, 13 in the cannibalism assay, and eight in the measure of surface-seeking behavior. Although there may be some common environment effects for each vial the use of several vials for each experiment eliminates the possibility that our results are simply the result of common environment. These beetles and the corresponding controls at the age of 4 wk posteclosion were used in the following experiments to measure the effect of high prevalence of parasitism on emigration rate, cannibalism, and surface-seeking behavior. Different, and thus statistically independent, individuals were used in each experiment.

Emigration rate.—The emigration apparatus is illustrated in figure 1 (Prus 1963; Craig 1982; Goodnight 1990). Emigration behavior was assayed 2 wk after infection. Emigration rate was calculated as the proportion of emigrated beetles in a total of 32 beetles. Because previous studies have shown sex differences in emigration behavior (Wu 1981), the sexes were tested separately. There were two infection treatments, infected and uninfected, and two species treatments. Each treatment was replicated 10 times (two species × two infection treatments × two sexes × 10 replicates = 80 emigration measures). After measuring emigration, we randomly sampled 50 beetles from each species and sex of the infection treatments and dissected them to determine the intensity of parasitism and prevalence of parasitism.

Cannibalism and fecundity.—In flour beetles, cannibalism occurs when mobile life stages (adults and larvae) eat nonmobile life stages (eggs and pupae) (Park et al. 1965; Stevens and Mertz 1985). We assayed adult cannibalism of eggs because adult beetles are parasited by the tapeworm, whereas larvae are not, and adult cannibalism of eggs is thought to be more important in determining population size than adult cannibalism of pupae (Park et al. 1965). Therefore, any changes in the rate of cannibalism by infected adult beetles could have an important impact on the *Tribolium* population.

Because eggs are both oviposited and cannibalized during an assay, cannibalism and fecundity cannot be measured directly. By introducing a known number

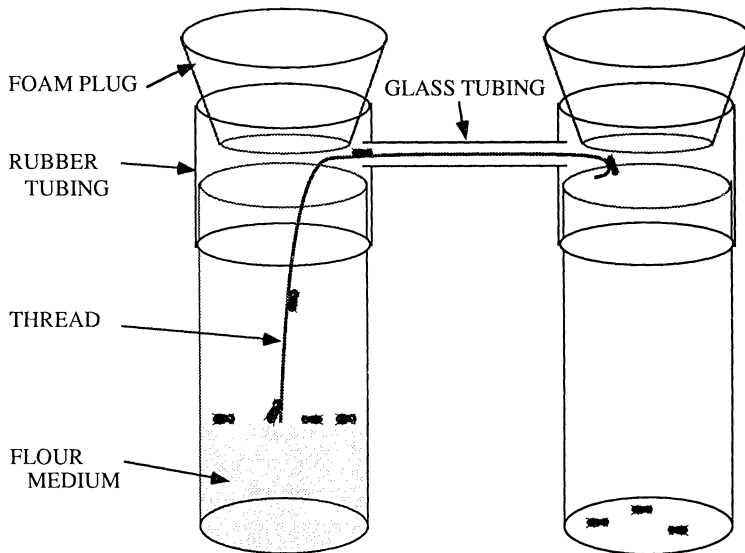


FIG. 1.—Two vials, one empty and the other containing 32 beetles and 8 g flour medium, are connected by rubber and glass tubing. A tiny thread suspended so that it just reaches the surface of the flour in the source population passes up through the tubing and into the second vial. The beetles climb up the thread, through the tube, and then fall into the second vial. Only one-way migration is permitted. The experiment is conducted in a darkened incubator. Emigration rate is measured as proportion of the 32 beetles in the second vial after 2 h.

of marked (i.e., dyed red) eggs and counting the number of both marked and unmarked (newly laid) eggs at the end of an assay period, the cannibalism rate, c , and real fecundity, E (eggs per female per day), can be estimated through the following equations (Rich 1956; Sonleitner 1961):

$$C = \frac{1}{bt} \log_e \frac{M_0}{M_t},$$

and

$$E = N_t C \frac{e^{bct}}{e^{bet} - 1} \left(\frac{b}{f} \right),$$

where b is the number of adult beetles, t is time in days, M_0 is the number of marked eggs introduced, M_t is the number of marked eggs recovered after t units, N_t is the number of unmarked eggs collected (net fecundity), and f is the number of females. The factor b/f converts real fecundity into eggs per female per day.

Cannibalism was assayed 2 wk after infection by placing 16 adults (sex ratio at unity) into a vial with 4 g of standard medium and 100 red-dyed conspecific eggs. The vials were placed in a dark incubator and cannibalism was assayed after 48 h by counting the number of marked and unmarked eggs in each vial. There

were two infection treatments and two species treatments. Each treatment was replicated 26–28 times.

Surface-seeking behavior.—We measured the number of beetles on the surface of the medium by putting eight male and eight female adult beetles into a vial with 8 g standard medium and placing it in a dark incubator. After 2 wk, we counted the number of beetles on the flour surface. There were two infection treatments and two species treatments. Each treatment was replicated 13–15 times.

Data analysis.—The data were analyzed for homogeneity and normality and transformed when appropriate (see table 1). We used the ANOVA in table 1A–E to test for the effect of parasitism on host emigration, cannibalism, real fecundity, net fecundity, and surface-seeking behavior. Orthogonal contrasts were used to compare within species and sexes the infection treatments with controls.

Host Behavioral Alterations under Low Infection Level

We examined the emigration behavior of six genetic strains of *T. confusum* (b+, b-Circle, bI, bIII, bIV, and bSM) and five genetic strains of *T. castaneum* (c+, cIII, cIV, c-Kyoto, and cSM) at low prevalence of parasitism (see Park et al. 1961, 1964, for the origins of b+, bI, bIII, bIV, c+, cIII, and cIV; Wade 1977 for bSM and cSM; Stevens 1989 for b-Circle; c-Kyoto was initiated in 1979 from 75–100 adults collected on rice from Kyoto, Japan [M. Wade, personal communication]).

Beetle infection.—Tapeworm eggs were recovered by conventional saturated NaCl floatation technique (Keymer and Anderson 1979). The egg concentration in the resulting suspension was usually about 200 per drop. Twenty adult beetles 7 d after eclosion were starved in a vial for 1 wk. Five drops (about 1,000 tapeworm eggs) of this mixture were placed on a 2 × 6.5-mm strip of filter paper and fed to the beetles for 24 h. After 24 h the filter paper was removed and the beetles were exposed to another mixture for 24 h. Then the filter paper was removed and 8 g of flour medium were added to each vial. One week later, the beetles were again starved for 1 wk. This infection process was repeated three times; therefore the beetles were exposed to six samples of parasite eggs. After we measured the emigration, 50–100 male and female beetles were randomly selected from each genetic strain and dissected to determine prevalence and intensity of parasitism. Control beetles were treated the same way as the infected beetles, except they were exposed to distilled water only.

Several factors probably combined to produce low prevalence of parasitism in this experiment. Although this method of infecting beetles is commonly used in laboratory studies, it resulted in a much lower prevalence and intensity of parasitism than did direct feeding of infected rat feces (G. Yan, unpublished data). Presumably the saturated salt floatation method resulted in a large proportion of inviable eggs (K. Boutte, personal communication). In addition, for the first two infections, microscopic inspection revealed a low egg density in the rat feces used to extract eggs and resulting solution (about 600 eggs in five drops of solution). After the first two infections, the beetles were relatively old and prevalence of parasitism decreased as beetles aged (Keymer 1981).

TABLE 1

ANOVA FOR PARASITE-INDUCED ALTERATIONS IN EMIGRATION, ADULT CANNIBALISM OF EGGS, NET FECUNDITY, REAL FECUNDITY, AND SURFACE-SEEKING BEHAVIOR

Experiment and Source of Variation	df	Mean square	F	P
High level of infection:				
A. Emigration: dependent variable is emigration rate:*				
Species	1	.0199	.9104	.3435
Sex	1	1.1372	51.9378	.0000
Species × sex	1	.3553	16.2275	.0001
Infection status	1	.0002	.0097	.9217
Species × infection status	1	.0993	4.5372	.0369
Sex × infection status	1	.0204	.9321	.3378
Species × sex × infection status	1	.2128	9.7189	.0027
Error	66	.0219		
B. Adult cannibalism of eggs: dependent variable is cannibalism rate:†				
Species	1	.0486	.1839	.6690
Infection status	1	.3933	1.4883	.2253
Species × infection status	1	1.3922	5.2689	.0238
Error	102	.2642		
C. Net fecundity: dependent variable is net fecundity:‡				
Species	1	2.0389	55.3037	.0000
Infection status	1	.1718	4.6603	.0333
Species × infection status	1	.2486	6.7432	.0108
Error	100	.0369		
D. Real fecundity: dependent variable is real fecundity:§				
Species	1	1.9796	37.0644	.0000
Infection status	1	.1026	1.9211	.1688
Species × infection status	1	.0971	1.8189	.1805
Error	100	.0534		
E. Surface-seeking behavior: dependent variable is surface beetles:				
Species	1	8.8460	36.1498	.0000
Infection status	1	.1142	.4666	.4975
Species × infection status	1	3.0031	12.2722	.0009
Error	54	.2447		
Low level of infection:				
F. Emigration: dependent variable is emigration rate:#				
Species	1	18.7654	24.5234	.0008
Strains[species]**	9	.7952	10.7062	.0086
Infection status	1	.0148	.4305	.5271
Species × infection status	1	.0397	1.1553	.3086
Infection status × strains[species]**	9	.0345	.8059	.6235
Sex	1	.3102	3.8533	.0803
Sex × species	1	.0018	.0229	.8830
Sex × strains[species]**	9	.0826	1.9280	.1711
Sex × infection status	1	.0395	.9345	.3576
Sex × species × infection status	1	.0150	.3559	.5647
Sex × infection status × strains[species]**	9	.0428	1.4547	.1669
Error	205	.0295		

Emigration rate.—The emigration apparatus illustrated in figure 1 was again used. There were two infection treatments: infected and uninfected; sexes were tested separately. Each treatment was replicated five to six times (two infection treatments \times two sexes \times 11 strains \times five or six replicates = 220–264 emigration vials). We tested the beetles' emigration behavior 2 wk and 4 wk after the last infection, when beetles were 9 wk and 11 wk posteclosion. After the second emigration trial, we randomly sampled beetles from the home and emigration vials of the infection treatments and dissected them to determine the intensity of parasitism and prevalence of parasitism.

Data analysis.—The arcsine transformation was used to make the variances homogeneous and to achieve normality. We used a repeated-measures ANOVA (SAS generalized linear model procedure for data with repeated measures; SAS 1985, pp. 433–506) and found no trial effect (ANOVA; $F = 0.67$, $df = 1$, 186, $P > .05$) and no interaction between trial and strain and sex and infection (ANOVA; $F = 1.51$, $df = 31$, 186, $P > .05$). We then averaged the emigration rate of two trials and used the ANOVA in table 1F to test for the effect of infection on host emigration. The strains were treated as a random effect. Orthogonal contrasts were used to compare, within species and sexes, the infection treatments with their noninfected counterparts.

RESULTS

Altered Behaviors under High Infection Levels

Beetle infection.—Generally, *Tribolium castaneum* was more susceptible than *Tribolium confusum* to infection with this parasite (Mann-Whitney U -test, $Z = 7.44$, $N = 100$, $P < .001$; see fig. 2). The average intensity of parasitism (mean number of individuals of tapeworm cysticercoids per host in a sample) of *T. castaneum* males was significantly higher than that of females (Mann-Whitney U -test, $Z = 3.62$, $N = 50$, $P < .001$) and the prevalence of parasitism of *T. castaneum* males was higher than that of females. *Tribolium confusum* males had a slightly higher mean intensity of infection and prevalence of parasitism than females, but the difference was not statistically significant (Mann-Whitney U -test, $Z = .87$, $N = 50$, $P > .05$ for intensity of infection).

NOTE.—In A–E the sources of variation are all fixed effects and the mean square error was used as the denominator for F -tests; F -tests in part F involve random effects. The denominators for each effect in the model were synthesized by the SAS JMP statistical program from linear combinations of the appropriate mean squares and have the same expectation as the effect to be tested under the null hypothesis. Degrees of freedom were adjusted for imbalance in the experiment by Satterthwaite's method (see SAS 1989, p. 409).

* Emigration data at a high level of infection are not transformed. Homogeneity test: $F_{\max} = 4.35$, $df = 8,9$, $P > .05$; normality test: Shapiro-Wilk $W = 0.987$, $P > .05$ (see SAS 1989, p. 228).

† Cannibalism data are natural-log transformed.

‡ Net fecundity data are natural-log transformed.

§ Real fecundity data are natural-log transformed.

|| Surface beetle data are transformed with square root (number of surface beetles + 0.5).

Emigration data at a low level of infection are arcsine transformed.

** The strains are nested within species and treated as a random effect.

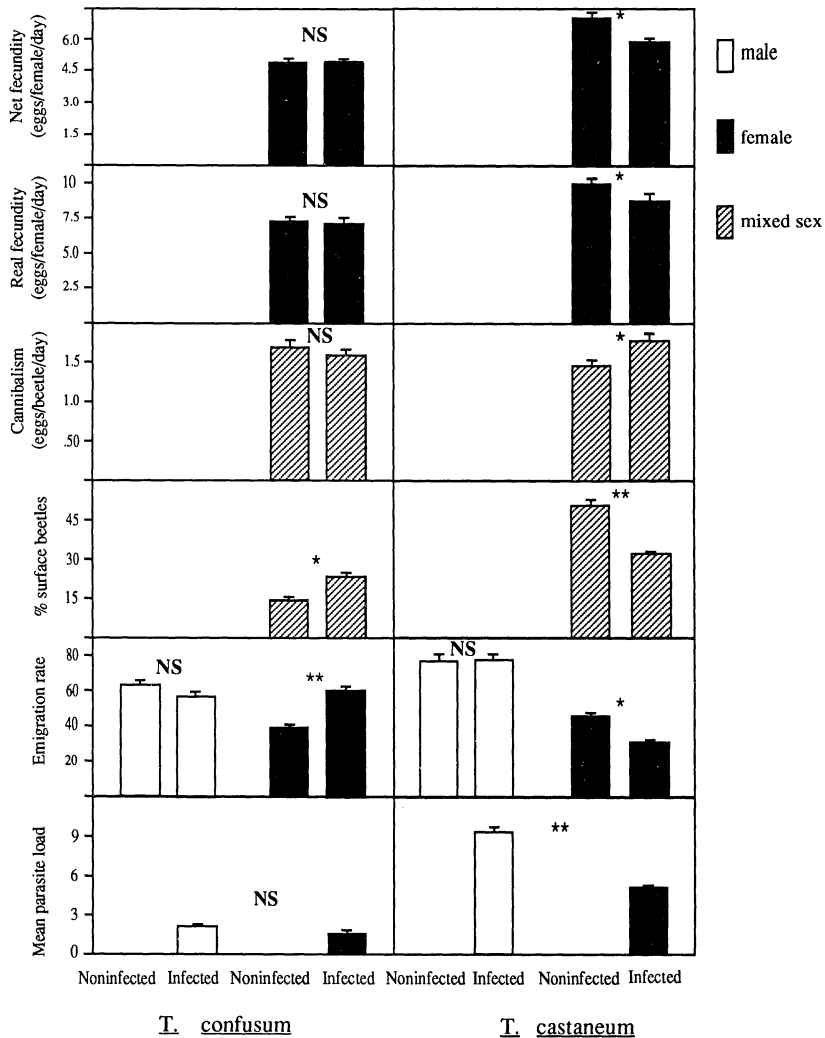


FIG. 2.—The tapeworm *Hymenolepis diminuta* induced alteration in three behavioral traits (emigration, surface-seeking behavior, and cannibalism) and one life-history trait (fecundity) in two species of flour beetles, *Tribolium confusum* and *Tribolium castaneum*. Data are presented as means + SEs. Prevalence of parasitism for *T. confusum* males is .72; for *T. confusum* females, .66; for *T. castaneum* males, .94; for *T. castaneum* females, .90; *, comparison is significantly different at $P < .05$; **, significant at $P < .01$; NS, not significantly different.

Emigration rate.—Parasite-induced effects on emigration behavior were species and sex dependent (ANOVA; $F = 9.72$, $df = 7,66$, $P < .01$ for species \times sex \times infection status; table 1A). Orthogonal contrasts demonstrated that parasites did not alter the emigration rate of male adults in either species. Infected *T. confusum* females showed almost twice the emigration rate as uninfected females (60.0% vs. 38.2%), and this increase was statistically significant (ANOVA orthogonal contrast; $t = 3.21$, $P < .01$; fig. 2). Parasites had the opposite effect on *T. castaneum*. For this species, the emigration rate of infected females decreased significantly from 45.7% to 31.3% (ANOVA orthogonal contrast; $t = 2.06$, $P < .05$; fig. 2).

Cannibalism and fecundity.—Infection significantly increased the cannibalism rate of *T. castaneum* from 1.37 eggs per beetle per day before infection to 1.67 eggs per beetle per day after infection (ANOVA orthogonal contrast; $t = 2.53$, $P < .05$). The cannibalism rate of *T. confusum* decreased to 1.19 eggs per beetle per day after infection from 1.37 eggs per beetle per day before infection, but this change was not significant (ANOVA orthogonal contrast; $t = .457$, $P > .05$; fig. 2).

The number of unmarked eggs collected (net fecundity) at the end of a cannibalism assay did not represent the real fecundity of *Tribolium* because some newly produced eggs were eaten. For example, the estimated real fecundities for uninfected *T. confusum* and *T. castaneum*, respectively, were about 44% and 36% larger than the net fecundity, owing to cannibalism (fig. 2). In *T. castaneum*, parasite infection significantly decreased both the real fecundity (10.0 eggs per female per day before infection vs. 8.83 after infection, ANOVA orthogonal contrast; $t = 1.99$, $P < .05$) and the observed fecundity (7.3 eggs per female per day before infection vs. 6.1 after infection, ANOVA orthogonal contrast; $t = 3.88$, $P < .001$). Infection did not change either the real fecundity or observed fecundity of *T. confusum* (ANOVA orthogonal contrast; $t = .026$, $P > .05$, and $t = .047$, $P > .05$, respectively).

Surface beetles.—Parasite-induced alterations of emigration behavior are related to changes in surface-seeking behavior. *Tribolium confusum* adults were more likely to be found on the surface of the medium when infected (ANOVA orthogonal contrast; $t = 2.03$, $P < .05$), whereas infected *T. castaneum* adults tended to hide inside the medium compared with uninfected individuals (ANOVA orthogonal contrast; $t = 2.91$, $P < .01$; fig. 2).

In summary, for *T. confusum*, tapeworm infection resulted in increased emigration of adult females, and this was related to an increased tendency to be on the surface of the medium. Parasitism did not significantly alter the rate of cannibalism or real or observed fecundity. In contrast, parasitism had an opposite effect on emigration and surface-seeking behavior in *T. castaneum*; parasitism decreased the emigration rate and the real and the observed fecundity of female adults, and these decreases were related to an increased tendency of infected adults to be in the medium and to an increased rate of cannibalism.

Behavioral Alterations under Low Infection Levels

Beetle infection.—Prevalence of parasitism varied significantly among genetic strains of *T. confusum* but not among genetic strains of *T. castaneum*. For *T.*

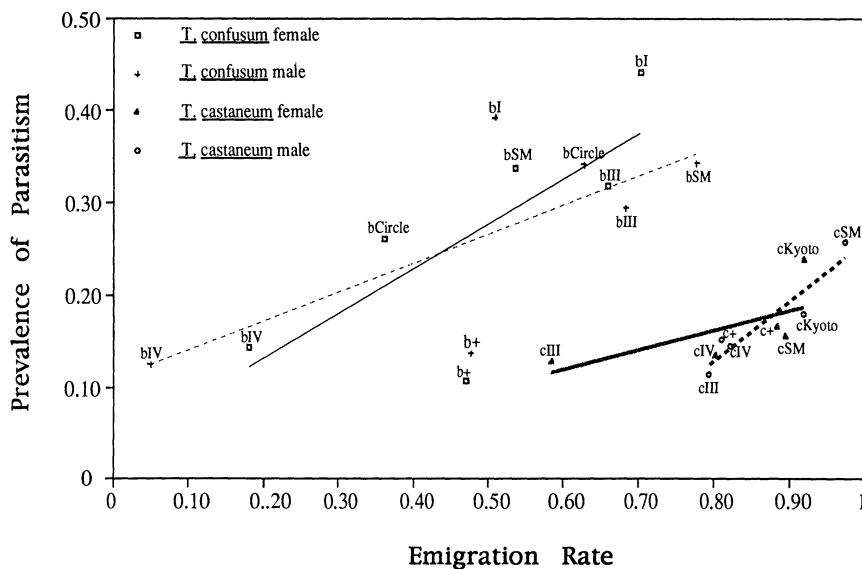


FIG. 3.—Correlation of prevalence of parasitism of six genetic strains of *Tribolium confusum* and five genetic strains of *Tribolium castaneum* with the mean emigration rate of uninfected beetles. Strain names are labeled. The correlation coefficients and significance levels are $r = .744$, $P = .090$ for *T. confusum* female (thin solid line); $r = .708$, $P = .116$ for *T. confusum* male (thin stippled line); $r = .663$, $P = .223$ for *T. castaneum* female (bold solid line); and $r = .939$, $P = .018$ for *T. castaneum* male (bold stippled line). The combined probability for all four correlations is less than .01 (see Sokal and Rohlf 1981, pp. 623–626).

confusum females, prevalence of parasitism ranged from 11% (b+) to 44% (bI) (G -test, $G = 31.142$, $df = 5$, $P < .01$; fig. 3), and the mean intensity of parasitism ranged from 0.11 (b+) to 0.87 (bI). Here mean intensity of parasitism is defined as the mean number of individuals of tapeworm larvae per host in a sample (Margolis et al. 1982). There was a high correlation between prevalence of parasitism and intensity of parasitism ($r = .94$, $N = 22$, $P < .001$; see the definition of prevalence and intensity of parasitism in the introductory section). For *T. confusum* males, the prevalence of parasitism ranged from 14% (b+) to 39% (bI) (G -test, $G = 25.882$, $df = 5$, $P < .01$; fig. 3) and the mean intensity of parasitism ranged from 0.16 (b+) to 0.72 (bI). For *T. castaneum* females, prevalence of parasitism ranged from 13% (cIII) to 24% (c-Kyoto) (G -test, $G = 4.858$, $df = 4$, $P > .05$; fig. 3), and the mean intensity of parasitism ranged from 0.13 (cIII) to 0.54 (c-Kyoto). For *T. castaneum* males, prevalence of parasitism ranged from 12% (cIII) to 26% (cSM) (G -test, $G = 7.038$, $df = 4$, $P > .05$; fig. 3), and the mean intensity of parasitism ranged from 0.14 (cIII) to 0.48 (cSM).

Emigration rate.—A comparison of uninfected beetles of 11 genetic strains revealed that, regardless of the genetic strain, *T. castaneum* showed a significantly higher emigration rate than *T. confusum* (ANOVA orthogonal contrast;

$t = 17.60$, $P < .001$; table 1F). Male adults had a higher but not statistically significant emigration rate than females for both *T. castaneum* (ANOVA orthogonal contrast; $t = 1.258$, $P > .05$) and *T. confusum* (ANOVA orthogonal contrast; $t = 0.883$, $P > .05$). Variation in the emigration rate among the genetic strains was significantly heterogeneous for both *T. castaneum* (ANOVA; $F = 18.419$, $df = 4$, 205 , $P < .001$; table 1F) and *T. confusum* (ANOVA; $F = 21.709$, $df = 5$, 205 , $P < .001$).

Infection did not significantly affect the emigration rate for any of the 22 comparisons examined (two sexes \times 11 strains) (ANOVA orthogonal contrast; $P > .05$ for all 22 comparisons). This suggests that this parasite does not affect emigration behavior at low level of infection.

Emigration Rate and Susceptibility to Parasitism

Examination of the results from both experiments suggested that the emigration rate of uninfected beetles and the susceptibility to parasitism were associated (susceptibility refers to the probability of acquiring parasites; it can be quantified with prevalence of parasitism or intensity of parasitism as defined in the introductory section). This could arise because more active hosts are more likely to encounter and consume parasite eggs and thus have higher risk of being parasitized. The high-infection experiment showed that uninfected males had higher emigration rates than did uninfected females (ANOVA orthogonal contrast; $t = 3.49$, $P < .001$ for *T. confusum*, and $t = 4.36$, $P < .001$ for *T. castaneum*; fig. 2). Even though *T. castaneum* male beetles were exposed to the same number of tapeworm eggs as were female beetles, the mean intensity of parasitism in male beetles was greater than in female beetles (Mann-Whitney *U*-test, $Z = 3.62$, $N = 50$, $P < .001$). In this instance high emigration rate was associated with a high probability of parasitism.

This possibility that the prevalence of parasitism was correlated to some innate activity level was investigated by analyzing the correlation between the emigration rate of uninfected beetles and the prevalence of parasitism for each sex for the six genetic strains of *T. confusum* and five genetic strains of *T. castaneum* (fig. 3; two species \times two sexes = four correlations, 5–6 replicates [genetic strains] per correlation). Because the correlations used emigration rate of uninfected hosts, we are testing whether there is a correlation between the innate activity level and the tendency to become infected. Although we had few degrees of freedom and thus little statistical power, one of four correlation coefficients was significantly greater than zero (*T. castaneum* males; $r = .939$, $df = 4$, $P = .018$) and all of the correlation coefficients were positive. These correlations were independent, so a combined probability test is appropriate (Sokal and Rohlf 1981, pp. 623–626) and showed that *Tribolium* activity was correlated with prevalence of parasitism (χ^2 test, $\chi^2 = 20.16$, $df = 8$, $P < .01$). This suggests that hosts with higher activity levels are more susceptible to the parasite. As prevalence of parasitism was significantly correlated with the mean intensity of parasitism ($r = .94$, $N = 22$, $P < .001$), both prevalence and intensity of infection were expected to increase with activity.

DISCUSSION

This article adds to the substantial evidence that parasitic infection can alter the behavior of hosts. What is the significance of such behavioral alterations? First, infection may disrupt courtship behavior of hosts, thus presumably reducing individual fitness. For example, male fence lizards (*Sceloporus occidentalis*) when infected with a malarial parasite appear unable to maintain a territory and compete with other males for access to females (Schall 1990). Second, parasites might alter their host's behavior in a way that is adaptive to the parasite by increasing its transmission success to the next host. Such scenarios could represent fascinating examples of coevolution of parasite and host but unfortunately are seldom supported by experimental evidence (Moore and Gotelli 1990). Third, altered behaviors and associated life-history changes may cause population and even community-level effects on their hosts: the host population size may be reduced or outcome of competition altered (Schall 1992).

Our study provides no information on effects of *Hymenolepis* larval infection on the mating behavior of *Tribolium* beetles. However, the changes we observed in cannibalism rate, fecundity, and migration rate induced in *Tribolium* by the tapeworm, and especially the different effects seen in the two beetle species, could well alter population size and competitive ability of the two species, similar to the ways a protozoan parasite shifts competitive outcome in two-species *Tribolium* experiments (Park 1948). The results, therefore, indicate *Hymenolepis diminuta* could play a significant role in the population and even community ecology of its beetle hosts. Finally, although our study shows the tapeworm alters the movement behavior of the beetles in ways that might affect transmission success of the parasite, the study actually provides a cautionary tale that demonstrates how easily data on behavioral changes in infected hosts could be misinterpreted as evidence that the trait is adaptive for the parasite.

We now review the evidence in support of these last two conclusions, beginning with our rejection of any adaptive manipulation of the beetles' behavior to increase transmission success of the parasite, followed by the evidence that this common parasite could cause swings in population size or competitive interactions in *Tribolium*.

There are many demonstrations of parasite-induced behavioral alterations in both vertebrate and invertebrate intermediate hosts. For example, pejerrey fish, *Basilichthys* spp., infected with the trematode *Diplostomum mordax* showed reduced activity and tended to stay on the water surface (Szidat 1969). Snakes (*Elaphe quadrivirgata*) infected with plerocercoids of the cestode *Spirometra erinacei* were less active (Fukase et al. 1986). Lemmings infected with the protozoan *Sarcocystis rauschorum* were less active and responded inappropriately to a threat from predatory snowy owls (Quinn et al. 1987). Mice, *Mus musculus*, infected with nematodes, *Trichinella spiralis*, reduced their ambulatory and exploratory open-field behavior and travel speed and distance (Rau 1983; Rau and Putter 1984). In invertebrates, the isopod *Armadillidium vulgare* infected with *Plagiohynchus cylindraceus* tended to be more active than uninfected animals (Moore 1983b). Cockroaches, *Periplaneta americana*, infected with *Moniliformis*

moniliformis increased activity both on a running wheel (Moore 1983a) and in open-field arenas (Wilson and Edwards 1986). *Tenebrio molitor* infected with the cestode *H. diminuta* were less active and photophobic (Hurd and Fogo 1991).

Most of these studies have been associated with hypotheses that parasite-induced behavioral alteration will facilitate parasite transmission by increasing the probability of the intermediate hosts being eaten by the appropriate definitive host. Note, though, that in some cases it is the reduced activity of the infected animal that is supposed to make them easier to catch by predators, whereas in the other cases it is increased activity that is argued to make the host more conspicuous to predators (Moore and Gotelli 1990). Information on the identity and foraging tactic of the common predators of each host would strengthen the "adaptive manipulation" hypothesis, but field studies on this problem are almost absent. For example, one of the best-known accounts of adaptive manipulation proposes that larvae of the sheep lancet fluke, *Dicrocoelium*, manipulate the behavior of infected ants to cause them to climb to the ends of grass blades and cease activity to make them more susceptible to ingestion by sheep. No experimental studies have been done to test this proposal, but field observations show that some ant species when infected with *Dicrocoelium* retreat into underground colonies, thus reducing the chance of parasite transmission to sheep (Moore and Gotelli 1990).

Our study showed increased movement in one species of host (*Tribolium confusum*) and decreased movement in the second (*Tribolium castaneum*) when they were infected with tapeworm larvae. These beetles occur in similar habitats in nature (Sokoloff 1972), the life cycle of the parasite is the same in both, and they are likely to share the same predators, so it is highly unlikely that opposite changes in their behavior could have similar results on transmission of the parasite to the next host. We noted that different genetic strains of the beetles exhibited different activity levels, and infection probability for the beetles is positively correlated with their activity level probably because the active beetles find more tapeworm eggs to eat. Therefore, the increased activity seen in infected *T. confusum* should result in infected beetles having even greater intensity of infection as they consume additional *H. diminuta* eggs. But, this result would be reversed in *T. castaneum*. Clearly, conclusions that parasites manipulate their host's behavior would best be supported by studies that consider phylogenetic relatedness and general ecology of the hosts (e.g., the studies on cockroaches and their acanthocephalan parasites [Allely et al. 1992; Gotelli and Moore 1992]). In our study, we conclude that the alterations seen in the behavior of *Tribolium* infected with larval tapeworms do not represent an evolved adaptation of the parasite.

Although the changes seen in the behavior of infected *Tribolium* seem unlikely to represent an adaptive manipulation of host behavior by the parasite, we do not conclude that these consequences of infection are what Dawkins (1990) facetiously calls a "boring" curiosity of parasite-host biology. The nature of the behavioral and reproductive changes in infected beetles suggests they may have an important influence on population dynamics and ecological interactions among species. Also, patterns in these ecological effects could be complex because of

at least four sources of variation in the alterations seen in infected beetles. First, the effects of infection differ for the two species of beetles. Second, infection with the larvae of *H. diminuta* altered the beetles' movement behavior in our experiments, but only when intensities of infection were high (for other examples of dose-dependent effects of infection on host behavior, see Schiefer et al. 1977, Rau 1983, Berry et al. 1986, and Webber et al. 1987). In our experiments, high intensities of infection were achieved by feeding moist rodent feces to starved beetles, a situation that might be unevenly distributed in nature. Third, the behavior of female beetles, but not males, was altered by infection with *H. diminuta*. This is probably driven by the kind of gender-specific physiological differences observed in *Tenebrio molitor* infected with *H. diminuta* larvae (Hurd and Arme 1984a, 1984b, 1987; Hurd and Parry 1991). Finally, infection rate differed among the genetic strains used in our experiments, suggesting that the mosaic of genotypes and genetically distinct populations in nature will vary in susceptibility to infection.

Correlations among the behaviors studied here also show that a direct effect of infection on one aspect of the biology of *Tribolium* can cascade into very broad consequences for the host. For example, we found that *T. castaneum* infected with *H. diminuta* was more cannibalistic. This might be related to the infection-induced change in emigration rate and surface-seeking behavior. Infected *T. castaneum* populations are more likely to be found inside the flour medium and thus more often encounter and cannibalize their own eggs. In contrast, tapeworm infection significantly increases the emigration rate of *T. confusum* female adults, and infected beetles tended to stay on the surface of the medium where emigration occurs, but eggs of the beetles are uncommon.

The increase in cannibalistic behavior and reduction in fecundity of infected *T. castaneum* should result in a decrease in the density of infected populations of these beetles. In contrast, such changes were not seen in *T. confusum*, which could put this species at an advantage when competing with *T. castaneum*. The problem for *T. castaneum* could be exacerbated by its tendency to be infected by larger numbers of tapeworm larvae. Thus, other costs of infection that we did not measure here, such as costs to life span, number of clutches of eggs per lifetime, and male reproductive success, could be more severe in *T. castaneum*.

In summary, *Tribolium* emigration was not altered by tapeworm infection under low infection levels. Under high level of infection, *T. confusum* and *T. castaneum* respond differently to infection in their emigration, cannibalism, and surface-seeking behavior. There is also genetic variation within species in prevalence of parasitism, intensity of infection, and behavioral responses to parasitism. This questions generalizations about behavior changes of several host species and even how genetic strains within a species are expected to respond to infection by a particular parasite. These results call for caution when proposing manipulation of host behavior by parasites. Even though they were exposed to the same number of tapeworm eggs, different genetic strains of *Tribolium* exhibited different infection rates. This suggests that genetic variation exists within species for susceptibility to infection and that susceptibility to infection could evolve as a result of future selection.

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