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Dynamics and Genetics of Mating Behavior in *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Three aspects of mating behavior—time to mating, repetitiveness, and mate selection—were investigated in six genetic strains of the flour beetle Tribolium castaneum *and their hybrids. The data were compared with predictions from theoretical models. Time to mating was found to be heritable and characteristic of a strain. In homotypic strain combinations the duration of mating was longer than in heterotypic combinations. In repeated matings the male mating rate declined with time. In a multiple-choice experiment there was a tendency for positive assortative mating; this did not occur in female-choice or male-choice tests. Keeping beetles of two strains together prior to the experiment removed this tendency. Males almost exclusively preferred virgin to fertilized females when given a choice. The data fitted Taylor's [(1975).* Behav. Genet. *5:381–393] model better than Kence and Bryant's [(1978).* Am. Nat. *112:1047–1062] model. The absence of assortative mating when males (and females) of two strains were held together can be explained by the pheromone-saturation model of Averhoff and Richardson [(1974).* Behav. Genet. *4:207–225] but not by Bryant's [(1979).* Behav. Genet. *9:249–256] alternative.*

KEY WORDS: *Tribolium castaneum*; Coleoptera; Tenebrionidae; mating time; mate selection; assortative mating.

INTRODUCTION

Mating behavior is an important component of fitness because it affects and modifies the contribution of different genotypes to the gene pool of

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succeeding generations. Mating speed, one important aspect of mating behavior in insects, is often used to estimate the competitive ability of strains in sterile-male pest control programs (e.g., Brower, 1978a,b). In studies of insect population dynamics it is often very important to predict the consequences of mating behavior on population size or genetic composition, and appropriate models are desirable. However, most behavioral work has been done in *Drosophila* (Ehrman and Parsons, 1976) and most theoretical models describing the dynamics of mating in insect populations are *Drosophila* oriented. The generality of these models is not always guaranteed. In addition, many models cannot be experimentally tested because they involve unmeasurable or otherwise undefined parameters (e.g., Kuno, 1978).

Flour beetles of the genus *Tribolium*, in particular *T. castaneum* and *T. confusum*, are convenient laboratory insects widely used in population ecological and genetical research (Sokoloff, 1966, 1972, 1974, 1977). Few studies of mating behavior in these species have been published (e.g., Wool, 1967; Sinnock, 1970; Taylor and Sokoloff, 1971; Rich, 1972), but these insects may be used conveniently to test theoretical models of mating behavior for applicability to organisms other than *Drosophila*. In fact, one theoretical model (Taylor, 1975) was developed on the basis of observations of mating in *Tribolium* and *Musca*.

The purpose of the present study was to quantify some components of mating behavior in genetically different *Tribolium* strains and their hybrids and to compare predictions from theoretical models with the observed results. Two of the models deal with the changes in frequency of unmated females with time (Taylor, 1975; Kence and Bryant, 1978) and are interesting from the point of view of population dynamics. Two other models (Averhoff and Richardson, 1974; Bryant, 1979) deal with the effects of inbreeding on mating behavior and are interesting for their implications for population genetics. However different these approaches may seem in theory, population ecology and population genetics cannot be separated in real populations.

MATERIALS AND METHODS

Six laboratory strains of *Tribolium castaneum*, five of them carrying morphologically distinct genetic markers, were used in this study. In addition, we reciprocally crossed four of the strains to a fifth. The strains and hybrids are listed in Table I. Strains are referred to below by the codes listed in Table I. In hybrids, the male parent code is listed first. In addition to the stock strains, we had two mutant strains, inbred black (IB) and inbred paddle (ID), that were propagated for nine generations by only one pair of adult sibs per line and generation. These were used to test the two models mentioned last.

Table I. List of Strains and Hybrids of *T. castaneum* Used in the Experiments.[a]

Strain name	Code	Genetic marker
bb	B	*b* (black body), linkage group III
paddle	D	*pd* (paddle-like antenna), X linked
pearl	E	*p* (pearl eye), linkage group II
red	R	*r* (red eye), linkage uncertain
pdbb	P	Synthetic, homozygous for both *pd* and *b*
CTC-12	C	Wild type (insecticide resistant) (Champ and Campbell-Brown, 1970)

[a] Additional details about the mutant strains are given by Sokoloff (1966, 1977). Hybrid codes are combinations of pure strain codes, male parent listed first.
Hybrids: BD, DB; BR, RB; BE, EB; and BC, CB. Inbred (I) strains (extracted after nine generations of sib mating): IB, ID.

Beetles for experiments were reared in a standard medium (flour and brewer's yeast, 20:1) under optimal conditions (30°C, 70% RH). Pupae were recovered and separated by sex, and emerging adults were held separately until use. Adults were at least 8 and no more than 77 days old when used in experiments. Preliminary tests convinced us that within this range, age had no effect on the mating parameters we measured ($P > 0.1$). Adults were used once only except in tests for preference between mated and virgin females (see below).

Three aspects of mating behavior were investigated.

Time to Mating

Mating was in 6.5 × 6.5-cm transparent plastic arenas with filter-paper floors. One or more pairs were introduced per arena without anesthesia, and the time from the introduction of a pair to copulation was recorded. Sixteen arenas could be observed simultaneously. The observation time was 30 min. All 14 possible homotypic combinations and 22 of 182 heterotypic combinations of strains were tested, in most cases 100 pairs per combination.

To learn something about heritable variation for mating speed within a strain, selection was practiced for two generations in strain B. B pairs were grouped by their times to mating as follows: 1–5 min (FA), 6–10 min (LE), 11–20 min (ME), and pairs which had not mated in 30 min (SL). A random sample of pairs (WS) was also taken from the stock. Ten pairs of each group were allowed to produce S_1 offspring which were tested at the adult stage. WS, FA, and SL were carried another generation and the S_2 offspring were also tested.

Repetitiveness

We define repetitiveness as the number of females a single male fertilizes in given time intervals. Groups of one male and nine virgin females, of the same or different strains, were introduced to vials with 1 g of medium for periods of 2, 4, 8, 16, 32, or 48 h. Each female was then transfered to a separate vial for 1 week. The number of inseminated females could easily be determined by the presence of larvae in the vials 2–3 weeks later. Four strains were tested for repetitiveness: B, D, DB, and BD (Table I).

Another estimate of repetitiveness was obtained when the female was replaced by a virgin after copulation was observed. The mated females were individually placed in vials with flour and fertilization was determined as above. The time to mating of the male with the first and subsequent females was also recorded.

Mate Selection (Mating Choice)

To test whether males preferred virgin over fertilized females, B males were simultaneously presented with two females, one of each type. When the male copulated with one of the females, the other was transferred to a vial with medium. Since unmated females do not produce larvae (although they do lay eggs), the presence of larvae in the vial indicated that the male selected the unfertilized mate. (The females used as "fertilized" all produced offspring in the days preceding the experiment.) By using this procedure we avoided the need to mark the females externally, which may induce biases in the results (Bryant *et al.*, 1980).

Male-choice and female-choice experiments were performed in arenas, pairing one beetle of one sex with two of the other (each of different strain). Multiple-choice experiments, with 10 pairs of each of two strains, were performed in petri dishes (d = 10 cm) with filter paper glued to the bottom. The observation time in the latter was 15 min only.

Yule's isolation index (Y) was calculated as by Petit *et al.* (1976) as $Y = (Z - 1)/(Z + 1)$, where $Z = (X_{11} \cdot X_{22}/X_{12} \cdot X_{21})^{1/2}$ and the X_{ij} values are the entries in the 2 × 2 table of mating frequencies. The standard error is $\frac{1}{2}(1 - Y^2)\sum\sum X_{ij}^{-1/2}$. Significance testing was performed with a chi-square test, using $\chi^2 = [\ln(Z)^2]^2/(\sum\sum X_{ij}^{-1})$. We also calculated male vigor (Mm) and female vigor (Mf) similarly.

RESULTS

Time to Mating

The means, standard errors, and coefficients of variation (CV) of time to mating for all homotypic and heterotypic combinations of males and females are listed in Table II, together with the number of pairs that copulated within 30 min (n) of the total number of pairs observed (N). Preliminary observations indicated that nearly all pairs would eventually mate if given longer periods of time (1 h or more) and no differences could be detected among strains. After 30 min, more than 50% of the pairs mated in the present experiment in all combinations. In some cases as many as 96% did (combinations 5 and 20). The variance among pairs within combinations was quite large (CV = 56–82%).

The data presented in Table II permit the testing of specific hypotheses. First, we wanted to know whether the male or the female genotype determines the speed of mating. An analysis of variance (ANOVA) indicated that the mean times to mating of B males to different females (combinations 1 and 15–20) were not the same (F = 5.86; df = 6,546; $P < 0.01$). Similarly, the times to mating of D males to different female genotypes (combinations 2, 21, 27, and 28) were not the same (F = 4.15; df = 3,286; $P < 0.01$). On the other hand, significant differences were also found when a D female (combinations 2, 15, 29, and 30) was mated to different male genotypes (F = 5.22; df = 3,298; $P < 0.01$). Similar differences were found when the common female strains were BD (combinations 7, 17, 27, and 36; F = 4.25; df = 3,245), RB (combinations 10, 20, and 32; F = 10.40; df = 2,230), and BR (combinations 9, 19, and 31; F = 7.45; df = 2,199). All these differences were significant at the 1% level. Testing for the contribution of interaction between males' and females' genotypes by means of a two-way ANOVA was not possible, but the data seem to suggest that the time to mating is affected by the interaction of both males' and females' genotypes.

The second question was whether the mating time in homotypic combinations was different from that in heterotypic combinations. In Table III, we group the mating combinations by male genotypes (A) and by female genotypes (B). Within each group the combinations are ranked by mating time, the *slowest* mating being ranked 1. The data reveal that homotypic matings are, on the average, slower than heterotypic matings in almost all male and female genotypes tested. The only exception is DB × DB, which ranked 4 (fastest for DB females). The differences within this group (combinations 8, 18, 28, and 35 in Table II), however, were not significant statistically (F = 0.93; df = 3,241). When all data are pooled, homotypic matings are significantly slower than heterotypic mat-

Table II. Times to Mating for Different Combinations of Males and Females[a]

(A) Homotypic combinations

	Strain	$\bar{y}$	SE	n	N	CV (%)
1	B	10.87	0.67	84	100	56.8
2	D	11.97	0.85	67	100	58.1
3	R	11.86	0.99	64	100	77.6
4	E	11.53	1.45	26	50	64.3
5	C	6.72	0.56	97	105	82.1
6	P	10.54	0.84	82	100	72.2
7	BD	10.28	0.84	83	100	74.8
8	DB	10.21	0.81	73	100	67.4
9	BR	12.76	1.02	54	100	63.2
10	RB	10.83	0.85	70	100	65.3
11	BE	7.19	0.70	43	50	63.8
12	EB	9.92	1.28	26	37	65.6
13	BC	9.44	0.45	204	234	67.9
14	CB	10.79	0.75	68	87	57.6

(B) Heterotypic combinations

	Male	Female	$\bar{y}$	SE	n	N	CV (%)
15	B	D	10.24	0.83	67	100	66.5
16	B	R	11.76	0.85	71	100	60.9
17	B	BD	8.81	0.69	75	100	67.9
18	B	DB	10.40	0.72	85	100	63.9
19	B	BR	8.40	0.62	75	90	63.8
20	B	RB	7.01	0.53	96	100	74.9
21	D	B	8.85	0.68	84	113	69.9
22	R	B	10.55	0.92	62	100	68.6
23	BD	B	8.00	0.69	87	100	80.1
24	DB	B	9.97	0.83	68	100	69.0
25	BR	B	9.97	0.84	71	100	70.8
26	RB	B	9.27	0.75	81	100	73.1
27	D	BD	11.83	0.85	69	101	59.8
28	D	DB	11.94	0.81	70	100	56.3
29	BD	D	8.57	0.62	84	100	66.3
30	DB	D	8.40	0.62	84	100	68.1
31	R	BR	10.88	0.77	73	100	60.7
32	R	RB	10.49	0.72	67	100	56.4
33	BR	R	11.52	1.02	60	100	68.6
34	RB	R	11.41	0.86	58	100	57.6
35	BD	DB	10.47	2.10	17	25	78.5
36	DB	BD	6.59	1.10	22	25	82.6

[a] n, number of pairs copulating in 30 min of N pairs observed. CV, coefficient of variation of time to mating within combinations.

ings (U_s = 120, t_s = 2.91, $P < 0.005$ by Wilcoxon's two-sample test on the data in Table II).

The third question of interest was whether the time to mating is an inherited, strain-specific trait. The large variation in mating times within strains (Table II) indicates, as could be expected, that the trait may be polygenic or affected by nongenetic factors (or both). One way to find out whether there is heritable variation is to select for fast and slow mating.

Clearly the mean time to mating (Table IV; Fig. 1, left) increased in SL as compared to WS in response to selection in both F_1 and F_2 (t = 2.76 and t = 2.18 respectively; $P < 0.05$). The means of FA, LE, and ME remained the same as in the nonselected line. However, the percentage of copulating pairs (Fig. 1, right) increased in FA (t = 2.10; $P < 0.05$), indicating that this line did respond to selection (if, on the av-

Table III. Ranks of Times to Mating in Homotypic and Heterotypic Combinations of Males and Females[a]

(A) Common male genotype	Female: B	D	R	BD	DB	BR	RB
B	2*	4	1	5	3	6	7
D	4	1*	—	3	2	—	—
R	3	—	1*	—	—	2	4
BD	4	3	—	2*	1	—	—
DB	2	3	—	4	1*	—	—
BR	3	—	2	—	—	1*	—
RB	3	—	1	—	—	—	2*

(B) Common female genotype	Males: B	D	R	BD	DB	BR	RB
D	2	1*	—	3	4	—	—
BD	3	1	—	2*	4	—	—
BR	3	—	2	—	—	1*	—
RB	3	—	2	—	—	—	1*
B	1*	6	2	7	4	3	5
R	2	—	1*	—	—	3	4
DB	3	1	—	2	4*	—	—

[a] Data are grouped by male genotype (A) and female genotype (B). The times to mating in each line were ranked, with the slowest given rank 1. Homotypic matings are marked with a asterisk. For data above the line, the differences within are statistically significant. A dash indicates that no data were available.

Table IV. Selection for Fast and Slow Mating in Strain B[a]

Generation	Line	$\bar{y}$	SE	n	N
P		9.2	1.2	32	39
S_1	FA	9.2	1.1	28	30
	LE	9.3	1.7	25	30
	ME	10.8	1.3	24	30
	SL	14.6	1.6	23	30
	WS	9.8	1.6	25	30
S_2	FA	9.0	1.1	29	30
	SL	13.5	1.6	20	30
	WS	9.7	1.7	26	30

[a] Line codes: FA, fast; SL, slow; WS, without selection; LE and ME, intermediate in mating time in the parent generation. N and n as in Table II.

erage, mating become faster, more pairs should have copulated in 30 min than before).

Repetitiveness

The presence of larvae was used as an indication of insemination in tests of repetitiveness. This is a convenient and relevant measure of insemination rate, because a fertile *Tribolium* female lays eggs for extended periods and is bound to produce offspring in the weeks between mating and inspection of the vials. Less than 8% of all females in random samples from the stocks (where all females are assumed to be inseminated) were sterile, and no difference was found among strains. Sterility in males could not be directly detected, and thus when none of the nine females was inseminated, we assumed that the male was sterile and repeated the experiment.

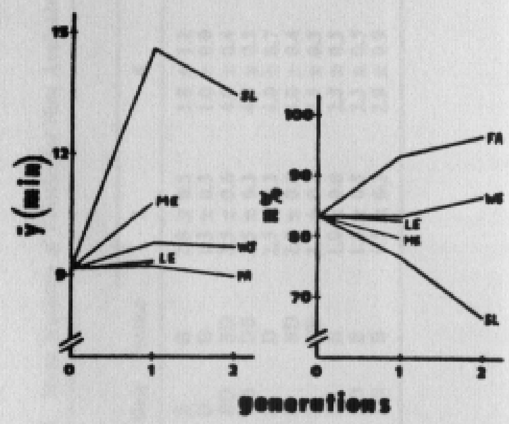

Fig. 1. Selection for fast (FA) and slow (SL) mating times in strain B. WS, no selection. LE and ME were intermediate in mating time in the parent generation (see text). $\bar{y}$, mean time to mating. n%, percentage of pairs mated.

The mean numbers of inseminated females, of the nine available in each strain combination, are listed in Table V. A two-way analysis of variance indicated differences among duration times and also among strain combinations, with no interaction, indicating that the change with time in the number of inseminated females is the same in all combinations. When combinations of different females with the same male were extracted from Table V (combinations 1 and 5–7, 2 and 8, 3 and 9, and 4 and 10) and tested separately by means of a two-way ANOVA without replications, no differences were found among combinations ($P > 0.05$), but when the data were rearranged and tested for differences among male types mated with the same female (combinations 1 and 8–10, 2 and 5, 3 and 6, and 4 and 7), these were highly significant ($P < 0.01$). Repetitiveness appears to be a property of male behavior only, and does not depend on the female genotype.

Table V and Fig. 2 illustrate that the insemination rate was not constant, but decreased in extended mating periods more than expected owing to loss of virgins, which follows an exponential distribution. The rate of insemination could decrease owing to two causes: (a) physiological fatigue or depletion of sperm and (b) a change in the average mating time. No change was found in the mean mating time of males exposed to three females consecutively (9.3 ± 1.4, 9.6 ± 1.8, and 9.2 ± 1.0; N = 50). We do not have direct evidence to point to sperm depletion as the major

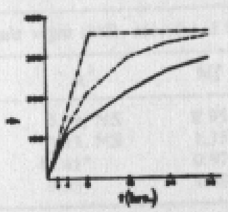

Fig. 2. Numbers of fertilized females in repetitiveness experiments; 360 females were available in groups of 9 to single males for each time period (all strains pooled). Solid line, observed results. Dashed line, expectation based on the (constant) mating rate of the first 2 h. Dashed–dotted line, a model assuming absolute preference of virgin females (see Discussion).

Table V. Mean Numbers of Females (of Nine Available) Inseminated by Single Males During Various Time Intervals

No.	Male	Female	Duration of experiment (h) 2	4	8	16	24	32	48
1	B	B	2.8 ± 0.5	3.8 ± 1.2	3.5 ± 0.6	6.5 ± 0.6	6.3 ± 0.5	8.0 ± 0.4	—
2	D	D	1.3 ± 0.3	1.0 ± 0.0	2.5 ± 0.3	3.5 ± 0.9	6.5 ± 0.6	8.5 ± 0.3	—
3	BD	BD	2.5 ± 0.6	4.0 ± 0.4	4.0 ± 0.4	6.3 ± 0.3	8.0 ± 0.4	8.0 ± 0.7	—
4	DB	DB	2.5 ± 0.3	4.3 ± 0.5	4.3 ± 0.5	5.0 ± 1.0	7.5 ± 0.3	7.0 ± 0.4	—
5	B	D	2.5 ± 0.3	4.0 ± 0.7	3.0 ± 0.7	5.0 ± 1.0	6.0 ± 0.8	5.5 ± 0.5	—
6	B	BD	2.0 ± 0.6	3.0 ± 0.4	5.0 ± 0.7	5.8 ± 0.5	7.0 ± 0.4	8.5 ± 0.5	—
7	B	DB	2.5 ± 0.3	2.5 ± 0.3	4.0 ± 1.1	8.0 ± 0.0	7.0 ± 0.4	8.0 ± 0.6	8.8 ± 0.0
8	D	B	1.0 ± 0.0	2.5 ± 0.5	3.5 ± 1.2	4.5 ± 1.3	5.8 ± 1.1	6.3 ± 0.9	8.5 ± 0.0
9	BD	B	1.5 ± 0.5	2.3 ± 0.5	3.5 ± 0.3	5.0 ± 0.7	5.5 ± 0.5	7.3 ± 0.5	—
10	DB	B	1.5 ± 0.3	2.8 ± 0.9	3.8 ± 0.6	4.8 ± 0.3	7.0 ± 0.7	7.3 ± 0.5	—

cause of the decrease in insemintion rate; however, we did find a slight—though not significant—decrease in the proportion of females fertilized in consecutive matings with the same male (80, 76, and 72%).

Mating-Choice Experiments

Males of *T. castaneum* were found to prefer virgin females almost exclusively when given a choice between a virgin and a fertilized mate. Of 59 such observations, the males chose the virgin female in 57; the assumption of no preference rejected, $\chi^2 = 51.27$, $P \ll 0.01$.

Male-choice and female-choice experiments were performed with strains B, D, and P taken two at a time in all possible combinations. No difference from the expected 1 : 1 ratio of mating with the two alternatives was found ($P > 0.05$ by χ^2 tests). There was no preference for either homotypic or heterotypic mates.

Table VI. Results of Multiple-Choice Experiments with D and B Beetles[a]

I. Assortative mating

	N	Number of mating between strains (male listed first) B × B	B × D	D × B	D × D	Y	χ^2
(a) Stock strains held apart	74	24	10	12	28	0.406	12.121*
(b) Stock strains held together for 2 weeks before mating	100	29	23	28	30	0.075	0.614, NS
(c) Inbred strains (IB and ID)	75	33	34	4	4	−0.007	0.001, NS
(d). Pure strains vs. hybrid (B and BD)	84	17	11	37	19	−0.058	0.233, NS

II. Male vigor (Mm) and female vigor (Mf), calculated by Merrell's (1950) formula

	Mm	χ^2	Mf	χ^2
(a)	0.85	0.49, NS	0.95	0.05, NS
(b)	1.08	0.33, NS	1.33	0.15, NS
(c)	8.38	46.41*	0.97	0.01, NS
(d)	0.50	9.33*	1.80	6.86**

[a] N, total number of matings observed. Y, Yule's index. Significance tested by χ^2 with 1 df.
* $P < 0.05$.
** $P < 0.01$.

In female-choice experiments, where two males were available, 19 of 167 recorded mounts were homosexual (11.4%). No strain differences in the frequency of homosexual mounts were detected. There is genetic variation for homosexual propensity in *Tribolium* (Rich, 1972).

However, in multiple-choice experiments mating was not random, and there was a clear tendency for assortative mating among strains ($P < 0.001$; Table VI, Ia). No differences between strains in male or female mating frequencies could be detected (Table VI, IIa).

When the inbred strains, IB and ID, were used instead of the pure stock strains, no assortative mating was detected, but there was a significant difference between the mating frequencies of males, the IB males mating much more often than the ID (Table VI, Ic and IIc).

Holding the males, and the females, of the two stock strains together for 2 weeks prior to the experiments canceled all traces of assortative mating, and there was no indication of differences between strains in male or female mating frequencies (Table VI, Ib and IIb). Finally, when B was tested against the F_1 hybrid, BD, no assortative mating was noticeable but both males and females differed significantly in mating frequency (Table VI, Id and IId). The most conspicuous effect was that BD males mated more often than B males (cf. Wool, 1970).

DISCUSSION

Mating patterns connect genotype frequencies at the end of one generation with offspring frequencies in the beginning of the next. Direct observation of mating frequencies among genotypes may provide information of predictive value.

In *Tribolium* it is impossible to observe mating in its "natural" habitat, within the flour. Problems associated with direct observations of mating were pointed out before (Wool, 1967), but results of such observations may correspond with predictions based on genetic studies (Wool, 1970).

No visible pattern of courting is detectable in *Tribolium* (Sokoloff, 1974). In an organism living under the surface of the medium, visual stimuli are unlikely to play a major role in detecting mates. It is possible that communication among mates is olfactory (McDonald and Spencer, 1964; Ryan and O'Ceallachain, 1976). In fact, *Tribolium* males will attempt copulation with dead females or other males (Wool, 1967), but the frequency of correct choice (in the present experiments, nearly 90%; see Mating-Choice Experiments under Results) indicates that they do discriminate between proper and improper mates.

The response to selection indicates that time to mating is a heritable

character. Parent–offspring correlation of mating times was 0.95. This is, however, an overestimate of heritability since the data from all parents and offspring were pooled within each line. Nevertheless, the additive genetic component seems large. It is interesting to note that time to mating was found to be highly heritable in such different species as *Drosophila melanogaster* (Parsons, 1964a, b) and *Gallus domesticus* (Siegel, 1972; Siegel and Cook, 1975).

The similarity of mating times of reciprocal hybrids (DB and BD, RB and BR) to each other and to their parent strains indicates no evidence of maternal effects, sex linkage, or heterosis. Heterotic effects, however, could be obscured by the slow mating in homotypic combinations.

Homotypic matings in this study occurred at a slower rate than heterotypic matings. Averhoff and Richardson (1974) presented a model to account for observations of mating behavior in *Drosophila melanogaster*. They suggested that every genotype produced characteristic cues which stimulate mating activity, but are not effective on the same genotype, because its chemoreceptors are saturated with its own odor. Inbreeding reduces variation in the population and then more and more individuals will produce the same odors and will not mate with each other, increasing the tendency for outbreeding. This can also explain the rare-male mating advantage (e.g., Ehrman, 1966; Ayala, 1972). The generality of the Averhoff and Richardson (1974) phenomenon was recently questioned when negative results were obtained in *Drosophila pseudoobscura* (Powell and Morton, 1979). Bryant (1979), following Kence and Bryant (1978), explained the same tendency of disassortative mating in inbred lines by assuming that male and female vigors decrease with inbreeding due to accumulation of low-vigor (slower-mating) homozygotes, with a corresponding increase in the homotypic mating time.

Averhoff and Richardson's (1974) model fits our data better than Bryant's (1979). Although the role of sex pheromones in *Tribolium* is unclear (Keville and Kannowski, 1975; Ryan and O'Ceallachain, 1976; O'Ceallachain and Ryan, 1977; Suzuki, 1979), holding males (and females) of two genotypes together for 2 weeks before mating removed the assortative mating between them (see Mating-Choice Experiments under Results) as if chemical strain-specific clues could no longer be used for discrimination between the strains owing to chemoreceptor saturation. These observations indicate that visual stimuli are not involved in finding mates, since the different strains carried clearly detectable morphological mutations. Similar results were obtained when *Drosophila melanogaster* and *Drosophila simulans* larvae were cultured together (Eoff, 1973).

Tribolium females may lay fertile eggs up to 200 days after a single copulation, but they mate repeatedly (e.g., Park, 1933) and repeated mating increases fecundity (Ruano and Orozco, 1966; Taylor and Sokoloff, 1971). Sperm precedence in *Tribolium* (Schlager, 1960; Vardell and Brower, 1978; Wool and Bergerson, 1979) gives advantage to males that copulate repeatedly, so selection for promiscuous male behavior may have taken place in the evolution of *Tribolium*. Another advantageous trait for males may have been to render the female unattractive after the first mating, at least temporarily. This strategy of ensuring paternity is known in many organisms (Giglioli and Mason, 1966; Gilbert, 1976; Ankney, 1977; Anderson and McGuire, 1978; Ross and Crews, 1978). Our observation that males almost exclusively preferred virgin females to inseminated females points in this direction, although the tendency for repeated matings may reduce the importance of such choice.

Comparisons with Theoretical Models

Two models were suggested for predicting the outcome of mating behavior in insect populations, which may be relevant for *Tribolium*. Taylor (1975) assumed that the outcome of mating can be predicted from knowledge of mating rate constants (α) characteristic of each genotypic combination in the population. These in turn could be calculated from the relation

$$dV_t/dt = -\alpha N_t V_t,$$

where N_t is the number of available males and V_t is the number of virgin females at time t. The parameter α depends upon two independent probabilities, the probability of an encounter between a male and a female and the probability that mating actually occurs after the encounter. More specifically, in *Tribolium*, pair formation was assumed to be of the "promiscuous" type, so that $N_t = N_0$ for every t (Taylor, 1975) and

$$V_t = V_0 e^{-N_0 t},$$

where V_0 is the initial number of virgin females.

Kence and Bryant (1978) offered another model, developed for *Musca* and *Drosophila* but assumed to work for *Tribolium* as well. They assumed that the probability of mating depends on the net difference, E, between stimulatory and inhibitory signals (vigor) of the potential partners. The time to mating is inversely related to $E(t = 1/E)$ and depends on the distribution of individuals of different vigor in the population. If individuals of different genotypes meet, some of the courtship stimuli may be inappropriate, so t will increase indefinitely in extreme cases, giving total reproductive isolation.

Each of the two models is necessarily based on assumptions about characteristics of mating behavior, although not all of them are explicitly stated. To decide which model is better for *Tribolium*, we discuss the suitability of some of the assumptions and compare some of the predictions of each model to our experimental data.

Taylor's Model

(1) *Tribolium* males are promiscuous. Our data support this assumption. The copulation duration is very short compared to the time to mating. For instance, for a random group of 16 B pairs, the mean copulation duration was 1.8 ± 0.3 min and the mean time to mating was 7.8 ± 1.6 min. The number of available males is, thus, expected to remain constant.

(2) Mating is random; virgin and mated females are equally likely to mate. This assumption is definitely not met by our data—males almost exclusively chose virgin females (see Mating-Choice Experiments under Results). This, however, had no apparent effect on the mean time to mating (see above).

(3) The parameter α is constant for every genotype combination and is time independent. Figure 2 illustrates that the ability of a male to fertilize females decreases with time. The total number of females fertilized was lower than expected on the assumption of a constant rate, in particular when virgin females are preferred (compare dashed–dotted line to solid line in Fig. 2).

(4) The number of matings is proportional to the number of male–female encounters. The probability of encounter, in an environment with fixed dimensions, depends on the perimeter/area ratio (Wool and Graur, in preparation) and should increase with the insect density: we observed mating at two densities, 2 and 20 pairs per arena. Five hundred pairs were observed at the high density and 122 at the low. The proportion of mated pairs after 15 min was significantly higher at the high density (51.6% at the high density, 37.7% at the low).

Kence and Bryant's Model

(1) The model rests on the concept of male and female *vigor*, which is not defined, except to say that the definition is not that of Bösiger (1958). It is implied that vigor is the sum of stimulatory and inhibitory signals of the mates, but it is not possible to measure these independently of courtship itself.

(2) **Male vigor and female vigor have opposite effects on the time to mating; a high male vigor increases the mating speed, but a high female vigor decreases it.** When this is true, the fastest matings should occur between males with the highest vigor (aggressive, persistent) and females with low vigor (passive, low tendency for rejection of a mate). The absence of visible courting in *Tribolium* does not permit evaluation of this assumption. In other species the assumption may be true. For example, a high social standing facilitates mating in roosters but reduces it in hens (Guhl and Warren, 1946; Guhl, 1950).

(3) Computer simulation suggested that the expected distribution of mating times in a population should be leptokurtic and skewed to the right (Kence and Bryant, 1978). This assumption is necessary for the differences in vigor to determine the mating speed. Experimental results in *Drosophila* support this assumption (Manning, 1961; Kessler, 1968; Spiess, 1968). In all 36 strain combinations in the present study, time to mating was skewed to the right (significantly so in 28). It appears, however, that this may be an artifact since the mating time is always positive, and as the mean becomes lower (faster mating), the distribution becomes both skewed and leptokurtic. A significant negative correlation between the mean, $\bar{y}_i$, and the statistics of skewness and kurtosis, G_1 and G_2, was found ($P < 0.05$; Figs. 3a and b). Detection of skewness and kurtosis of mating times cannot be used to support Kence and Bryant's (1978) model.

(4) In homotypic, single-pair matings (mean vigor of males and females assumed to be equal) the proportion of encounters ending in fertilization should be about 50% (encounters in which the female's vigor will be larger than the male's will not result in copulation). Neither our data (Table II) nor some *Drosophila* observations (Connolly *et al.*, 1974) support this prediction. Similarly, our data do not support the prediction that times to mating should be negatively correlated in reciprocal strain combinations, because a high-vigor male would mate quickly if the female has a low vigor, therefore mating would be very slow if the genotypes are reversed. The correlation was far from significant ($r = -0.023$).

Agreement between predictions from a theoretical model and experimental results does not mean that all the assumptions on which the model is based are true. Taylor's model emphasizes the probability of encounter and is basically stochastic. Kence and Bryant's model emphasizes the processes during courting, between encounter and actual copulation, and is basically deterministic. The former model seems to fit *Tribolium* better, mainly because no detectable courting behavior seems to exist, although not all its assumptions and predictions are supported by our study.

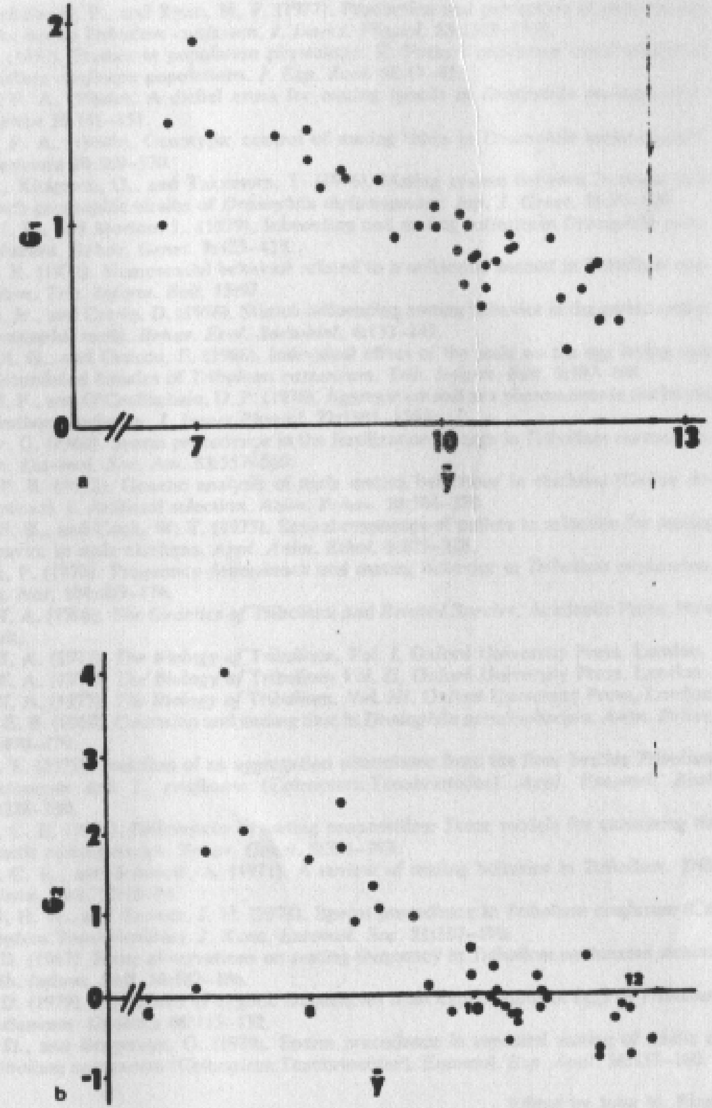

Fig. 3. Correlation between mean time to mating ($\bar{y}$) and (a) skewness, G_1, and (b) kurtosis, G_2, in the 36 mating combinations.

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