

Unidirectional interspecific mating in *Tribolium castaneum* and *T. confusum*: evolutionary and ecological implications

Dan Graur¹ & David Wool

Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, P.O. Box 39040, Ramat Aviv, Tel Aviv 69978, Israel

¹ Present address: Center for Demographic and Population Genetics, University of Texas Health Science Center at Houston, P.O. Box 20334, Houston, TX 77225, USA

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Abstract

Interspecific mating between two species of *Tribolium* occurs only between males of *T. confusum* and females of *T. castaneum*. The reciprocal mating combination was not observed. This pattern of unidirectional mating resulted in a frequency-independent reduction in the number of inseminated females in mixed populations in comparison with single-species mating groups. The ecological significance in terms of interspecific competition, and the possibility of identifying evolutionarily ancestral and derived species from such data are discussed.

Introduction

Closely related species of some insects which cohabit sympatrically, including flour beetles of the genus *Tribolium*, are known to interact with each other on several levels. These interactions seem to affect profoundly various components of fitness, like energy utilization (Bowker, 1979), fecundity (Birch *et al.*, 1951) and facilitation and developmental rate (Dawson, 1964). Because sexual isolation is a primary mechanism in sympatric speciation (Dobzhansky, 1970), one of the more surprising forms of interaction between competing species is the occurrence of interspecific mating. Most students of behavior tend to regard mating as a stereotyped form of behavior whose function is to ensure conspecific matings, and as a provision against energy losses due to interspecific and, hence, infertile mating attempts. However, sterile interspecific matings do occur (Strong & Arndt, 1962; Vick, 1973), and these have the potential to affect greatly the outcome of interspecific competition; thus, determining to a great extent the popula-

tion make-up of a habitat.

Tribolium castaneum (Herbst) and *T. confusum* DuVal (Coleoptera: Tenebrionidae) may occur in the same habitats, and, thus, potentially compete for the same resources (Birch *et al.*, 1951; Dawson, 1964; Vardell & Brower, 1976; Bowker, 1979). The purpose of the present investigation was to determine the effect of interspecific matings on the results of competition between *T. castaneum* and *T. confusum*. In addition, we wished to determine, in species other than *Drosophila*, whether or not the direction of species evolution can be inferred from unidirectional matings (Kaneshiro, 1976; Watanabe & Kawanishi, 1979; Markow, 1981; Giddings & Templeton, 1983). This study was part of a larger scale investigation on *Tribolium* mating behavior (Graur & Wool, 1982).

Materials and methods

The laboratory strains of *Tribolium castaneum* (CS) and *T. confusum* (CF) were black (Sokoloff *et*

al., 1960) and *Chicago* (Chapman, 1924), respectively. Beetles were reared in standard medium (flour and Brewer's yeast, 20:1) at near-optimal conditions (30 °C, 70% r.h.). Pupae were recovered and separated by sex, and emerging adults were held separately until use. Two aspects of mating were investigated: time to mating and the proportion of inseminated females during a fixed observation period in pure- and mixed-species groups.

Mating was observed directly in 6.5 × 6.5 cm plastic arenas with filter paper floors. For measurement of the first variable, one pair was introduced per arena, the female first. The time from introduction of the male to copulation was recorded. A maximal time of 30 min was allowed. This period was previously found sufficient for up to 90% of *CS* pairs to mate (Graur & Wool, 1982). Sixteen arenas could be observed simultaneously. All four possible reciprocal combinations of species were observed. For the second variable, groups of forty beetles (20 ♂♂ and 20 ♀♀) in different combinations of the two species (frequencies of *CS*: 0, 10, 20, 50, 80, 90, 100%) were introduced into a vial with 1 g medium for 4 h. Each female was then transferred to an individual holding vial with medium for 1 week and then discarded. The presence of larvae in these vials 2–3 weeks later, indicated that the female was fertilized by a conspecific male.

Results and discussion

Mean time to copulation and the proportion of mating pairs in 30 min in the four combinations are listed in Table 1. Mean time to mating was found not to differ significantly between the conspecific matings of *CS* and *CF*, but four times as many *CS* pairs mated in 30 min as *CF*. In heterospecific combinations, *CF* males were found to mate equally

Table 1. Mean time to mating (T) and the proportion of mating pairs.

♂	♀	T ± S.E.	% Mated	N
<i>CS</i>	<i>CS</i>	10.9 ± 0.8	84	100
<i>CS</i>	<i>CF</i>	–	0	25
<i>CF</i>	<i>CS</i>	10.2 ± 1.2	20	25
<i>CF</i>	<i>CF</i>	12.1 ± 1.0	20	50

N = numbers of pairs tested.

Table 2. Experimental design and the proportion of inseminated ♀♀ in pure and mixed cultures of *T. castaneum* and *T. confusum*.

% <i>CS</i>	No. replicas	Proportion of ♀♀		Proportion of inseminated ♀♀ (%) ± S.E.	
		<i>CS</i>	<i>CF</i>	<i>CS</i>	<i>CF</i>
0	5	0	100	–	78 ± 2
10	5	10	90	70 ± 12	30 ± 1
20	3	12	48	50 ± 0	40 ± 2
50	4	40	40	53 ± 3	38 ± 3
80	3	48	12	75 ± 3	42 ± 8
90	5	90	10	69 ± 1	30 ± 12
100	5	100	0	90 ± 4	–

Forty beetles (20 ♂♂ and 20 ♀♀) per replica.

successfully with either *CS* or *CF* females. Neither time to copulation (*t* test, $P > 0.05$) nor proportion of mating pairs in 30 min (χ^2 test, $P > 0.05$) were significantly different in these combinations. On the other hand, none of the 25 *CS* males ever attempted copulation with a *CF* female, even when the observation time was extended beyond 30 min (the probability of such occurrence by chance, assuming equal mating speed in interspecific and intraspecific matings, is less than 2%). This unidirectional interspecific mating in *Tribolium* may explain the large drop in productivity in heterospecific matings between *CS* females and *CF* males, in comparison with the relatively minor drop in productivity in the reciprocal heterospecific combination (Sokoloff & Inouye, 1963; Sokoloff & Lanier, 1967).

The results of the second experiment are shown in Table 2. The proportion of inseminated females of both species was always lower in mixed-species than in single-species groups, independent of the proportions of the two species coexisting in the same vial. No significant difference was found between the number of inseminated *CS* and *CF* females in single-species groups. In mixed groups, a significant difference (χ^2 test, $P < 0.001$) was found between the proportion of inseminated *CS* and *CF* females, with more *CS* females inseminated by conspecific males in all combinations.

Two conflicting hypotheses have been proposed to infer the evolutionary sequence on the basis of differences in the degree of success in reciprocal matings between closely related species. Watanabe & Kawanishi (1979) predicted that females of a

derived species would not mate with males of the ancestral species, whereas females of the ancestral species would readily mate with males of the derived species. Their rationale is that during the initial stages of a sympatric speciation event the newly evolved species would have a small population size, and, consequently, if the newly evolved females mate with males of the original population, depletion of the new population results. But if a male of the new population mates with a female of the original population, he may mate again conspecifically, and the new population is not adversely affected. This assumption is valid for most *Drosophila* species where females mate only once but males mate more than once. In *Tribolium*, although females mate more than once, we recorded a definite preference for virgin females in choice experiments (296 out of 360 males chose the virgin female, $\chi^2 = 149.5$, $P < 0.001$), and we, thus, believe that the premises of Watanabe and Kawanishi's model are also applicable to *Tribolium*, at least as an approximation. Kaneshiro (1976) essentially predicted the opposite trend, albeit, his model is expected to work better in reference to allopatric speciation. Unfortunately, the experimental evidence as to which theory is correct, if either, and under which conditions, is conflicting (e.g. Kaneshiro, 1976; Ohta, 1978; Watanabe & Kawanishi, 1979; Wasserman & Koepfer, 1980; Markow, 1981; Dwivedi *et al.*, 1982; Moodie, 1982; for a recent opinion and a review see Giddings & Templeton, 1983). Because the evolution of *Tribolium* does not follow in all details Kaneshiro's assumptions, we can only test Watanabe and Kawanishi's hypothesis.

Based on cytological evidence, Smith (1952a, b) suggested that *CF* was derived from *CS* by translocation of one of the autosomes of the ancestral form ($n = 9$ autosomes + X + Y) to the X chromosome, yielding the present complement of *CF* ($n = 8$ autosomes + a larger X + Y). If these conclusions are valid, then our results agree perfectly with the predictions derived from Watanabe and Kawanishi's theory, namely, females of the derived species (*CF*) do not mate with males of the ancestral species (*CS*), while the reciprocal matings occur at frequencies comparable to conspecific matings. This conclusion, however, depends heavily on the veracity of Smith's phylogeny, and there may be reasons to doubt it (Sokoloff, 1966; Wool, 1982).

Ecologically, both gains and losses are possible from interspecific matings (miscegenation) between the two *Tribolium* species used in this study. One species, for instance, may gain resources otherwise shared with the competing species's offspring, by interfering with the other species' mating process and reducing the fertility and fecundity of its females. Conversely, interspecific matings may be costly, at least for males, in terms of time and energy spent on infertile matings, thereby reducing reproductive success and, consequently, competitive ability (we have observed that the percentage of females inseminated by a male upon consecutive matings decreases while the time to mating remains the same, indicating that males experience some depletion of sperm).

Assuming sufficient evolutionary time (Hinton (1948) places the *Tribolium* speciation event in the Cretaceous), if interspecific mating evolved (or persisted) and was not selected against, overall gains must have exceeded the losses. In the case of *CF* males it is not clear how the balance between gains and losses is attained. While the activity of *CF* males causes a reduction in the number of inseminated *CS* females, the proportion of inseminated *CF* females was even more strongly affected in the mixed cultures. Copulation of *CF* males with *CS* females is known to cause serious genital damage to the latter. Marked reductions in fecundity, egg fertility and progeny number occur in later conspecific copulations (Lloyd & Park, 1962). However, *CS* males did not cause significant damage to *CF* females in the very few cases where miscegenation of this type was observed (Sokoloff, 1966). Indeed, our results indicate that mating strategy follows these premises: whereas *CF* males showed equal tendency to mate with females of both species, *CS* males did not at all attempt copulation with *CF* females. This behavioral tendency of *CS* males is striking in view of its otherwise indiscriminate mating practices (Woo, 1967; Graur & Wool, 1982). This unidirectional interspecific mating pattern explains the observed reduction in the number of fertilized *CS* females in mixed cultures. We tentatively attribute the even greater reduction observed in *CF* females to slower mating speed (Park, 1934; Stanley & Grundmann, 1965; Wool, 1967), slowed down even further by the engagement of *CF* males in interspecific and homosexual matings (McDonald & Spencer, 1964; Wool, 1967), and to the lower fertili-

ty of *CF* compared with *CS* (Wool, 1967). Because *CS* has higher fecundity, faster mating and shorter generation time than *CF*, it seems that the tendency of *CF* males to mate equally frequent with females of both species has provided *CF* with a mechanism against complete elimination from mixed populations in its 'natural' habitat, man-made flour and grain stores.

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Résumé

Accouplements non réciproques entre Tribolium castaneum et T. confusum: conséquences évolutives et écologiques

Les accouplements interspécifiques entre deux espèces de *Tribolium* n'ont lieu qu'entre mâles de *T. confusum* et femelles de *T. castaneum*. Les combinaisons réciproques n'ont pas été observées. Ce type d'accouplement asymétrique a provoqué une réduction indépendante de la fréquence dans le nombre de femelles inséminées dans les populations par rapport aux groupes d'accouplements monospécifiques. La discussion porte sur la signification écologique dans la compétition interspécifique et les possibilités d'identification des espèces ancestrales et dérivées.

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