

application of a technique which can be used in the analyses of cytoplasmically inherited phenomena. Our results show that a maternally inherited difference in mtDNA is associated with the Texas male-sterile cytoplasm. These observations suggest that the factors conditioning cytoplasmic male sterility and the cytoplasmic inheritance of susceptibility to *B. maydis* and *P. maydis* are located on the mitochondrial genome. Although we cannot disregard chloroplasts or other cytoplasmic DNA's as potential carriers of these traits, the preferential effect of the host-specific fungal toxins on mitochondria from cms T lines (5), together with the restriction endonuclease data, constitute strong evidence that the mitochondrion is the organelle involved in the inheritance of the traits.

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10. The RI endonuclease from *E. coli* was a gift from M. Edgell, University of North Carolina, Chapel Hill. The RI restriction buffer contained 0.1M tris(hydroxymethyl)aminomethane-hydrochloride (tris-HCl), 5 mM MgCl<sub>2</sub>, and 50 mM NaCl (pH 7.5). Digestions were at 37°C for 1 hour.  
11. The dIII endonuclease from *H. influenzae* was purchased from Miles Laboratories, Inc. The dIII restriction buffer contained 6 mM tris-HCl, 6 mM MgCl<sub>2</sub>, 50 mM NaCl (pH 7.5), plus 5 µg of bovine serum albumin per 50 µl of digestion volume. Digestions were at 37°C for 1 hour.  
12. The agarose gel electrophoresis and staining procedures were similar to those of M. Thomas and R. W. Davis [*J. Mol. Biol.* **91**, 315 (1975)] except that ethidium bromide was not incorporated into the gels. Gels measured 0.5 to 0.6 cm by 12 to 16 cm; a constant voltage of 1.5 to 1.8 volt/cm was applied.  
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Aggression and Mating Success in Male Spider Mites

Abstract. *Male Tetranychus urticae search for and defend quiescent pharate females. Intruding males may be threatened or attacked. Fights involve pushing and grappling with the forelegs, jousting with the mouthparts, and entangling the opponent with silk. In these encounters larger males usually win. Sole possession of a female at her ecdysis virtually ensures successful mating.*

In polygynous mating systems the ability to conquer or intimidate other males is an important component of male fitness (1). Despite the relative paucity of analytical studies of invertebrates, it is evident that sexual selection generates aggressive interactions within many groups of arthropods (2). Agonistic behavior has been reported in male spider mites (3, 4), but its significance has not been recognized. We found that aggressive interactions between competing males of *Tetranychus urticae* Koch are frequent and that they determine the ultimate success or failure in mating.

Spider mites are phytophagous colonizing organisms with a short life cycle (minimum, 10 days) (5) and a high intrinsic rate of increase (6). In *T. urticae* the tertiary sex ratio is generally 3 : 1 (female to male) (6). While males are sexually capable throughout their adult lives, normally only teneral virgins are available as potential mates. As a consequence, the functional sex ratio is skewed toward males. Sperm from the first mating has precedence (7); therefore, competition for mates is intense and male aggression is important.

The mites used in our study were from the Ohio State University strain and the Sambucus strain (wild type and albino). The two genotypes of the Sambucus strain are sexually compatible and mating is nonassortative (8). Cultures were

maintained on kidney bean plants and observations were made on isolates on 12- or 17-mm bean leaf disks pressed onto wet cotton and kept under constant light at 27° ± 2°C.

Female deutonymphs (penultimate instar) spin strands of silk, settle on the leaf surface, and become quiescent during their final moult. Wandering adult males, guided by the silk webbing (9) and a sex attractant (4), discover the quiescent females. These females become increasingly attractive over time (8), and as ecdysis approaches virtually all are attended. Males assume a characteristic guarding position, resting on the dorsum of the female with their forelegs across her body.

Other males, on encountering the pair, often attempt to climb on top of the female. If the first male fails to respond to the intrusion, the two become co-guarders, vying for the preferred position on the female. Usually, however, the resident male does not tolerate the intruder and, if the challenger is persistent, a fight ensues. Agonistic encounters can be characterized, in order of increasing intensity and decreasing frequency, as follows: (i) intruder retreating without aggression; (ii) one-sided aggressive response or threat, usually by the guarding male, ending with retreat of intruder; (iii) moderate to intense aggressive interaction involving both males and ending

Table 1. Size and success of male *Tetranychus urticae* in aggressive encounters.

Character measured	Number of fights in which winner was:			$\chi^2(1d.f.)^*$
	Larger	Equal size	Smaller	
Length of tarsus 1	13	2	3	6.25 ( $P = .012$ )
Length of tarsus 4	16	1	1	13.24 ( $P = .0003$ )
Distance between prodorsals II	11	2	3	4.57 ( $P = .033$ )

\*Cases of equal size are not considered.

with retreat of one; and (iv) intense fighting resulting in injury to, or death of, one combatant.

A fight may be initiated by either individual by confronting the other with outspread front tarsi. The challenged male either backs away or responds by raising and spreading apart his forelegs and extruding his cheliceral stylets.

The combatants circle and rush each other, flailing their forelegs and jousting with their extruded stylets. There is much pushing and grappling. Mites often use their palpal glands to apply strands of silk to the mouthparts and legs of the opponent. An individual may be so badly entangled that his movements are impeded and he retreats to clean himself, thus ending the encounter, at least temporarily.

Rarely, one male punctures the other's integument with his stylets. Males injured in this way are crippled and death usually results. Although very few agonistic encounters of the fourth type were observed in progress, dead deflated males were not unusual in the vicinity of guarded females.

Size is important in fighting. We selected 20 decisive fights and compared winners and losers with regard to size of three length characters (Table 1). On the basis of these criteria, winning males are consistently larger.

A successful combatant returns to the preferred position on top of the quiescent female. The male in possession of the female at her ecdysis will have the first opportunity to mate with her. If a resident male is unsuccessful in driving away intruders he is either replaced or he becomes one of two or more co-guarders at the female's ecdysis and competes for access to the female when she emerges. The reproductive advantage of solitary guarding was measured. Seventy active albino (a recessive trait) female deutonymphs were placed alone on leaf disks. After the females became quiescent (at 6 hours), a single male, alternately albino and wild type, was introduced on the disk (at 12 hours). Later (at 24 hours), a second male of the opposite genotype was placed on each disk. The relationship between the males and the quiescent female was checked every half-hour until the female's emergence, and all instances of guarding and co-guarding were recorded. After ecdysis and mating, the females were left to oviposit and the males were removed. The progeny were reared, enabling us to determine their sex and phenotype. Albino daughters indicated a mating with an albino male; wild-type daughters resulted from a wild-type father. Males who guarded

alone during the final half-hour before ecdysis were almost always successful in mating (93 percent). Males who occupied the preferred position on top of the female but tolerated co-guarders had a reduced frequency of mating success (63 percent) (10).

It is likely that, within a spider mite colony, male aggression does not generate any specific dominance order but rather gives rise to a random series of confrontations. Guarding pharate females represents a form of ephemeral territoriality; the procurement and retention of such a territory is to a large extent dependent on fighting ability.

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10. Mating success of single guarders does not differ significantly from an expectation of 100 percent ( $\chi^2 = 0.91$ , d.f. = 1,  $P = .340$ ); that of co-guarders who occupied the preferred position did not differ significantly from an expectation of 50 percent ( $\chi^2 = 0.13$ , d.f. = 1,  $P = .718$ ).
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## Genetic Predisposition and Stress-Induced Hypertension

**Abstract.** *When chronically exposed to an approach-avoidance conflict, rats with a genetic susceptibility to hypertension showed persistent elevations in systolic blood pressure, but rats with a genetic resistance to hypertension did not. Hence, psychic stress is selectively efficacious in producing hypertensive effects depending on genetic predisposition of the animal.*

The etiologic significance of psychic stress in hypertension is still controversial. However, despite some conflicting results and methodological difficulties, animal experiments indicate that appropriate psychic stress can result in sustained but usually moderate increases in blood pressure with associated anatomical pathological changes (1). A neglected factor in previous research is the genetic predisposition of the experimental animals. The absence of reported attempts to investigate the interaction of psychic stress and genetic predisposition is surprising, especially since most stress studies have been done on rats, the same animal used in developing genetic models for hypertension.

Dahl *et al.* (2) developed two strains of rats in this laboratory by selective inbreeding of Sprague-Dawley rats according to their blood pressure response to salt ingestion. The Dahl hypertension-sensitive strain (S) rapidly and predictably develops severe fatal hypertension upon excess salt ingestion whereas the Dahl hypertension-resistant strain (R) responds mildly or not at all. Using this rat model Dahl's group found that other putative hypertensinogenic stimuli were also selectively efficacious depending on genetic predisposition (2). Unlike the spontaneously hypertensive rat (3), S

strain rats remain normotensive when not exposed to a specific hypertensinogenic stimulus.

Despite their genetic predisposition, when S rats were exposed to aversive Pavlovian conditioning procedures—that is, electric shocks were administered independent of the subjects' behavior—no hypertension resulted (4). However, exposure of S rats to an aversive operant conditioning schedule involving a food-shock conflict did result in persistent, occasionally severe elevations in blood pressure. The food-shock conflict also resulted in severe food deprivation and frequent electric shocks. When S rats were exposed to identical amounts and patterns of either food deprivation or electric shocks or both but independent of their behavior, they did not exhibit blood pressure elevations as persistent or severe as those of the conflict-exposed rats (5). This indicates that exposure to conflict is more hypertensinogenic than simple exposure to aversive events.

In the experiment reported here, both S and R rats were exposed to the food-shock conflict in order to determine whether the effects of this type of stress depend on genetic predisposition. Beginning approximately 3 weeks after weaning, male rats were exposed either 5 or 7