Odorous Secretion of Normal and Mutant Tribolium confusum

Abstract. *An autosomal recessive mutant characterized by "melanotic stink glands" has been found in the tenebrionid flour beetle,* Tribolium confusum. *The contents of the reservoirs of both pairs of odoriferous glands become modified in two ways as the beetles age. (i) The quinones (2-ethyl- and 2-methyl-1,4-benzoquinone) present in the secretion are reduced to 1/20 of that normally found in the wild type. (ii) The contents may be modified into a high-molecular-weight polymeric substance, which becomes visible through the exoskeleton and, upon dissection, appears as a black solid lump. The medium in densely populated cultures of the mutant becomes moldy while that containing normal beetles remains particulate. This difference suggests that one of the functions of the secretions of the odoriferous glands is to prevent the growth of fungi or bacteria in the nutrient flour.*

There are two pairs of odoriferous or stink glands in at least four species of tenebrionid flour beetles in the genus *Tribolium* (*T. anaphe, T. castaneum, T. confusum*, and *T. destructor*); one pair is located in the prothorax and another in the abdomen (*1*). As the normal adults emerge from the pupae, the glands secrete a yellowish fluid which is stored in special reservoirs. The fluid has been analyzed in both *T. castaneum* and in *T. confusum*. In *T. castaneum* it consists of 2-ethyl-,2-methyl-, and 2-methoxy-1,4-benzoquinones (*2*), and an oily secretion whose molecular weight is 179 (*3*). In *T. confusum* only the 2-ethyl- and 2-methyl-1,4-benzoquinones were present (*4, 5*).

The quinones are discharged when the beetles are excited, and even in open, deep vessels the beetles may cause their own death in the absence of flour. In contact with air a tacky black substance of unknown nature forms on the abdomen of these moribund beetles. Exposure of pre-imaginal forms (at critical stages in their development) to the gaseous quinones resulted in various teratological abnormalities in the adults metamorphosing from them. Similar deformities were obtained when larvae or pupae were exposed to hydrochloric or glacial acetic acids in liquid or gaseous form (*6*).

When flour beetles are placed in a confined environment with a limited food supply, their feeding and metabolic activities (including the discharge of quinones) alter the flour, and the flour acquires a pinkish color, an offensive odor, and a disgusting taste. The flour is said to be "conditioned." This conditioning has different effects on fecundity and other biological attributes of both *T. castaneum* (*7*) and *T. confusum*.

We now report the differences between a mutant strain and the normal wild-type *T. confusum* in the content of the reservoirs of the odoriferous glands and the odorous secretions. Normally, in the wild type, the content of the reservoirs is a yellowish fluid not visible through the red-rust pigment of the exoskeleton. As the beetles become senescent, the reservoirs become visible, because the contents become dark red, but they remain fluid. These beetles are still capable of discharging quinones when irritated. In the "melanotic stink glands" mutant (*msg*), beetles only a few weeks old have reservoirs containing black material more or less similar to the shape of the reservoirs, or the material may be divided into several small masses (*8*). Furthermore, upon dissection, the contents of the reservoirs seem crystalline when touched with forceps. If fluid remains in the reservoirs, it is colorless and unlike that found in normal beetles upon dissection of their reservoirs. The *msg*-mutant condition results from the action of an autosomal recessive gene linked with, and about 42 units away from, the body-color gene black (*8*).

Isolation of the odorous secretion of the beetles was carried out by placing the beetles (from 125 to 10,000 beetles per experiment) on the center plate of the apparatus (Fig. 1). A fairly fast stream of dry air was passed through this vessel and then through two cold traps, the first of which was cooled in an ice bath and the second, containing a few milliliters of methylene dichloride, was cooled to −70°C. The chamber containing the beetles was immersed in ice water for 30 minutes; then the bath was removed, and the vessel was warmed moderately with a hair dryer for 15 minutes. This process was repeated three times over a total period of 3 hours.

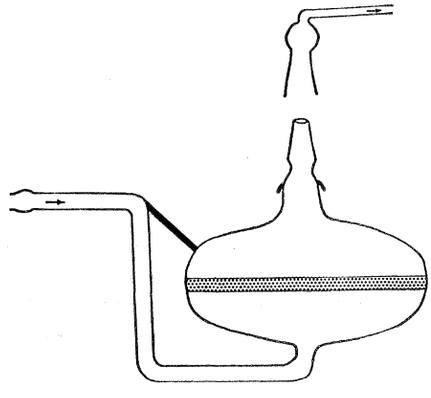

Fig. 1. Apparatus for collecting secretion of *T. confusum*. The center plate is a sintered-glass filter of medium porosity and the chamber is 6.5 cm across and 4.5 cm high.

Table 1. Composition of the odorous secretion of wild-type and *msg* mutant *Tribolium confusum*. The data are for young beetles. Old *msg* mutants gave no quinones, whereas age caused very little change in the wild-type secretion. The average weight of the beetles was 2.0 ± 0.4 mg.

Compound	Yield per 1000 beetles	
	Wild type (mg)	*msg* (mg)
2-Methyl-1,4-benzoquinone	3.3	0.15
2-Ethyl-1,4-benzoquinone	8.1	.45
2-Methylhydroquinone	0.01	
2-Ethylhydroquinone	.15	
Colorless oil, $C_{14}H_{20}O$	1.08	.97

When the foregoing procedure was applied to wild-type *T. confusum* yellow crystalline material and a colorless liquid (mostly water) had collected in the first trap, and a very small amount of yellow material had collected in the second trap. These were combined and subjected to vapor-phase chromatography (10 percent SE 30 on acid washed, dimethyldichlorosilane-treated Chromosorb W, 60/80 mesh, 1.5 m by 0.6 cm, 100°C). Easily separated and identified by comparison (of vapor-phase-chromatography retention times, ultraviolet and infrared absorption, and mass spectrometric fragmentation) with authentic samples were 2-ethyl-1,4-benzoquinone and 2-methyl-1,4-benzoquinone in the ratio 3 : 1. The 2-methoxy-1,4-benzoquinone was not found; and, considering the sensitivity of the isolation and analytical procedures, it must be absent. Small amounts of the corresponding hydroquinones were found as well as a colorless oil which will be described.

When the foregoing isolation procedure was applied to the *msg* mutant, no alkylquinones or hydroquinones could be detected. However, when young mutant beetles were used, very small amounts of 2-methyl- and 2-ethyl-1,4-benzoquinone were found and also the same colorless oil as was isolated from wild type.

The data on isolated secretion products for both wild-type and *msg* mutant *T. confusum* are compared in Table 1. Although both secreted 2-methyl- and 2-ethyl-1,4-benzoquinone, the amount obtained from the wild type was approximately 20 times greater in each case. From neither strain was 2-methoxy-1,4-benzoquinone detected, and only the wild type gave the corresponding alkylhydroquinones.

Both strains yielded the same amount of colorless (and relatively odorless) oil by this procedure. Although this substance was not completely identified, some information on its structure was obtained. This oil has the molecular formula $C_{14}H_{26}O$ (molecular weight 210 by mass spectrometry), showed a carbonyl absorption in the infrared at 1710 cm^{-1}, and $—CH_3$ (δ 1.1) and $=CH_2$ (δ 5.1) absorption in its nuclear resonance absorption. Since there was no aldehydic proton absorption, this compound appears to be an acyclic ketone with a terminal, nonconjugate (very weak ultraviolet absorption at 285 mμ) vinyl group.

The black material in the gland reservoirs of mutants is a high-molecular-weight, polymeric material. It appears to consist of polymerized quinones and may have arisen because the inhibitor of hydroquinone oxidation (*5*) is absent. The quinones thus may be formed prematurely and polymerize in the reservoir. This also is consistent with the much decreased amount of alkylquinone and absence of hydroquinone in the secretion of *msg*.

There has been much speculation about the role of the odorous secretions in *Tribolium*. They may have played a defensive role when *T. castaneum* and *T. confusum* occupied a different habitat; but as Roth (*1*) has emphasized, today *Tribolium* encounters few predators in the flour it infests, and quinones appear to be ineffective in warding off mites which may be the flour beetles' chief predators in their present habitat.

Van Wyk *et al.* (*9*) find that *T. confusum* is generally attracted to flour containing storage fungi or bacteria isolated from the beetles themselves, but as the population increases "the population of storage fungi decreases almost to the vanishing point, presumably because the quinones, secreted by the beetles, are toxic to the fungi." These authors believe that one of the functions of the malodorous secretion is to keep the food material relatively free of microorganisms which, if they were allowed to grow without restraint, would compete directly with, or make the substrate unsuitable for, the insect.

It would seem that Van Wyk *et al.* (*9*) are correct in ascribing such a function to the beetles' malodorous secretions. We observed that the medium in crowded cultures of normal beetles remained particulate, and did not become moldy; but in crowded cultures of the mutant, the medium developed a grey-green mold and became cakey. Also, the population of the mutant appeared to increase more rapidly than that of the wild type.

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Hydra: Induction of Supernumerary Heads by Isolated Neurosecretory Granules

Abstract. *Neurosecretory granules of* Hydra littoralis *were isolated by differential centrifugation. Excised midsegments of hydra that were exposed to the fraction containing neurosecretory granules developed additional heads at any site, as they regenerated. Hence neurosecretory granules may contain a factor that regulates or stimulates growth in normal and regenerating hydra.*

The nervous system of hydra appears to be necessary for regeneration because exposure to any one of several inhibitory neuropharmacological agents evokes abnormal regeneration or inhibits regeneration (*1*). At a fine structural level, some nerve cells in hydra contain dense, membrane-bounded granules, 1000 to 1200 Å in diameter, in the perikaryon especially in close relationship to the Golgi apparatus and in the neurites and their endings (*2*). After transection, these granules, presumably neurosecretory in nature, accumulate in the nerve terminations and are released into the intercellular spaces at the regenerating site (*3*). These observations suggest that a growth-stimulating or form-regulating factor may be located in the neurosecretory granules. In order to investigate this possibility further, neurosecretory granules were isolated by differential ultracentrifugation, and

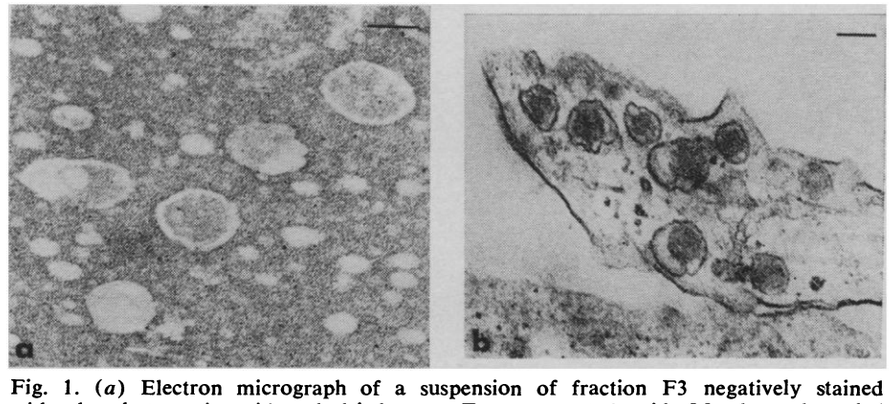

Fig. 1. (*a*) Electron micrograph of a suspension of fraction F3 negatively stained with phosphotungstic acid and dried on a Formvar-coated grid. Membrane-bounded granules, 1000 to 1200 Å in diameter, are present in addition to smaller particles. (*b*) Electron micrograph of a thin section of a neurosecretory cell neurite containing dense, membrane-bounded granules. Bars represent 0.1 μ.