Sex Peptide of Drosophila melanogaster

Abstract. All tested stocks of *Drosophila melanogaster* exhibit a peptide in males but not in females. The failure of some investigators to demonstrate its presence is attributable to their choice of chromatographic solvents. Acid hydrolysis confirms its peptide identity. Its presence in females transformed into males by the mutant *tra* implicates the sex-determining loci in its formation.

Chen (*1*) has recently reported failure to confirm our earlier reports of the chromatographic demonstration of a peptide among the free Ninhydrin-positive substances of adult *D. melanogaster* males and its absence in females (*2, 3*). In our work we had squashed whole flies directly on filter paper sheets and had performed two-dimensional, descending chromatography, using butanol : acetic acid : water as solvent in one dimension and phenol in the other. Chen, on the other hand, used extracts in 80 percent methanol and developed his chromatograms first with 70 percent *n*-propanol in the ascending direction and then with water-saturated phenol in the descending direction. The following simple experiment demonstrates that his failure to find the sex peptide is attributable to his choice of chromatographic solvents.

Four hundred males or females of an isogenic Oregon R stock were thoroughly ground in an all-glass homogenizer with 1.0 ml of each of the following solvents: 80 percent methanol, 80 percent ethanol, and water. The alcohol extracts were clarified by centrifugation. After initial centrifugation, the water extract was boiled for 2 minutes to denature proteins, and recentrifuged. Some (0.05 ml) of each extract was deposited on each of the chromatographic sheets (Whatman No. 1 filter paper, 46.5 by 57 cm), dried, and subjected to two-dimensional chromatography. Some sheets were developed according to the methods of Chen. Others were subjected to descending development in the first dimension (20 hours) with butanol : acetic acid : water (4 : 1 : 5 by volume), and in the second dimension (20 hours) with a solvent consisting of phenol (Merck) and 0.1*M* borate buffer (*p*H 8.3) in a ratio of 4 : 1 by volume. Ninhydrin-positive spots became visible on drying. Their identity had been established in previous work by means of cochromatography with pure amino acids (*4*).

Figure 1 depicts part of the results. In brief, the sex peptide is found in water, methanol, or ethanol extracts of males, but not in any of the extracts of females. When sheets are developed according to the methods of Chen, however, the peptide overlaps the spot produced by glutamine and could be overlooked even though its Ninhydrin color is somewhat more purple than that of glutamine. Its absence from the chromatograms of Kaplan *et al.* (*5*) may be explained in a similar fashion.

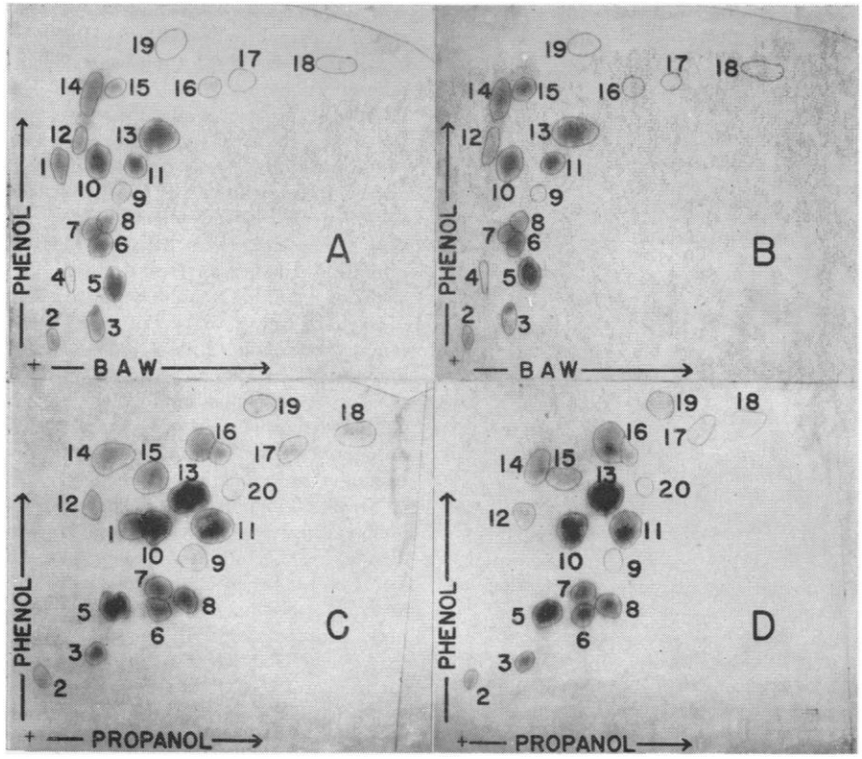

Fig. 1. Chromatographic separation of free Ninhydrin-positive substances of *D. melanogaster* extracted with 80 percent methanol. *A* and *C*, males. *B* and *D*, females. *A* and *B* were developed according to the method described in text; *C* and *D* were developed according to method of Chen (*1*). Identity of spots: 1, sex peptide; 2, peptide; 3, aspartic acid; 4, peptide; 5, glutamic acid; 6, serine; 7, taurine; 8, glycine; 9, threonine; 10, glutamine; 11, α-alanine; 12, lysine; 13, β-alanine; 14, arginine; 15, histidine; 16, methionine or methionine sulfoxide, or both; 17, valines; 18, leucines; 19, proline; 20, tyrosine.

When the appropriate portions of six or more sheets like that in Fig. 1*A* (not Ninhydrin-treated) are eluted with butanol : acetic acid : water (4 : 1 : 1) and the eluates are evaporated to dryness, sufficient sex peptide is obtained for further analysis. The products of hydrolysis of this material by concentrated HCl (110°C, 6 hours, followed by removal of HCl by evaporation) are water-soluble and yield four or five Ninhydrin-positive spots when subjected to chromatography. Our previous conclusion that this material is a peptide is thus confirmed.

We have now examined males of 16 different genotypes and females of 34 different genotypes, and in every case the sex peptide has been present in males and absent in females. This would indicate that this sexual difference is characteristic of the species as a whole, and it may also be true of other insects, particularly Diptera (*6*). We have previously demonstrated that this difference between males and females is attributable to the difference in number of X chromosomes (one in males, two in females) rather than to the presence and absence of the Y chromosome, thus suggesting the involvement of the sex-determining genes carried by the X chromosome (*3*). Examination (*7*) of the free Ninhydrin-positive substances of genetic females (2X/2A) transformed into sterile males by the third chromosome recessive mutant *transformer* (*tra, 8*) discloses that they possess the sex peptide. Since *tra* is a modifier of the genetic sex-determining system, the conclusion that the sex peptide is a product of that system is substantiated (*9, 10*).

Allen S. Fox
Charles G. Mead
Ilse L. Munyon

Department of Zoology,
Michigan State University,
East Lansing

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have prevented him from making the pertinent observations.

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Terminal Oxidase of Orchard Grass

Abstract. The response of infiltrated surviving green leaves to HCN, 1-phenyl-2-thiourea, and sodium diethyldithiocarbamate is consistent with a functional role for cytochrome oxidase in respiration. Polyphenol oxidase does not function as a terminal oxidase in orchard grass.

The identity of the functioning terminal oxidase in green leaves of grasses has not been clearly established. While studying losses of dry matter in the curing of hay, I therefore examined the terminal oxidase responsible for respiration in the leaves of green grass. Cytochrome oxidase has been reported in various plant tissues, but the pathway of electron transfer varies among different plants (*1*). Polyphenol oxidase has been regarded by many as a possible terminal oxidase, although there is little direct evidence of its primary function in undamaged cells (*2*). However, reports that it is at least partially functional continue to appear (*3*), so that it appears desirable to offer contrary evidence.

Daly *et al.* (*4*) reported that respiration of young leaves of barley was mediated by cytochrome oxidase. However, in older leaves respiration was not inhibited by carbon monoxide; thus the functional oxidase in mature leaves was left unknown. Deijs and his co-workers (*5*) showed that, as rye grass dried, its decline in respiration was paralleled by a similar decline in polyphenol oxidase activity. These workers attributed HCN inhibition of grass respiration to the effect of cyanide on polyphenol oxidase.

Orchard grass (var. Potomac) contains cytochrome oxidase and abundant amounts of polyphenol oxidase. Neither the intact leaf nor homogenates of it oxidizes ascorbic acid. Cytochrome oxidase appears to be the principal functioning terminal oxidase.

Grass of height 10 to 20 cm was homogenized for 40 seconds in a Waring blender with cold 0.1*M* phosphate (5 ml of buffer per gram of grass). For the assay of polyphenol oxidase the phosphate buffer was *p*H 6.5, for cytochrome oxidase, *p*H 6.8. Since addition of 0.2*M* sucrose and 0.001*M* ethylenediaminetetraacetate did not increase cytochrome oxidase activity, these compounds were usually omitted (*6*). For ascorbate oxidation, the homogenizing medium was 0.1*M* citrate-phosphate, *p*H 5.7 (*7*). Glass-distilled water was used throughout.

Cytochrome oxidase was manometrically measured in darkness at 30°C with 0.014*M* *p*-phenylenediamine as substrate. This enzyme is stimulated two- to threefold by the addition of $10^{-5}M$ exogenous cytochrome *c*. The most active fraction of cytochrome oxidase is sedimented in 20 minutes at 6230 *g* (average). For the assay of polyphenol oxidase, oxidation of catechol was followed manometrically (*8*). Polyphenol oxidase activity is not sedimented by 16,700 *g* in 20 minutes.

Several enzyme inhibitors were studied both in vitro and in surviving leaves. Grass was cut into pieces 1 to 2 cm long and vacuum infiltrated with inhibitors in 0.1*M* phosphate, plus 0.2*M* sucrose at *p*H 6.8, prior to respiration measurements. Table 1 shows that the effects of inhibitors in vivo are similar to those obtained with the cytochrome oxidase preparation but that they differ from those obtained with polyphenol oxidase. The stimulation of cytochrome oxidase and leaf respiration by diethyldithiocarbamate could be caused by its acting as a substrate for this oxidase (*9*). The response of intact green leaves to inhibitors is consistent with a functional role for cytochrome oxidase in respiration. Polyphenol oxidase cannot be the terminal oxidase in this issue.

Table 1. Effect of inhibitors on oxidases and on respiration of orchard grass.

Inhibitor	Percentage change in activity from control*		
	Polyphenol oxidase (in vitro)	Cytochrome oxidase (in vitro)	Infiltrated surviving leaves
$10^{-3}M$ HCN	− 90	− 100	− 40
1-phenyl-2-thiourea, saturated (*8*)	− 100	0	0
$10^{-3}M$ Na diethyldithiocarbamate	− 100	+ 16	+ 60

* The endogenous respiration of grass infiltrated with 0.1*M* phosphate, *p*H 6.8, averaged 270 μl of O_2 per hour per gram of fresh grass. Oxygen uptake per Warburg flask averaged 111 μl/hr for cytochrome oxidase, and 330 μl/hr for polyphenol oxidase.

HARVEY L. TOOKEY

Forage and Industrial Crops Laboratory, Northern Utilization Research and Development Division, U.S. Agricultural Research Service, Peoria, Illinois

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Transmission of Rabies to Laboratory Animals by Bite of a Naturally Infected Bat

Abstract. An insectivorous bat that attacked a man in western Montana was induced to bite suckling mice. Subsequently the bat died, and brain and salivary gland suspensions were inoculated into other mice. Rabies virus was isolated from all three groups of mice.

In a review of the relationship between bats and rabies, Enright (*1*) noted that transmission of rabies by bite of insectivorous bats had not been demonstrated. Burns (*2*) failed to obtain transmission to monkeys, guinea pigs, and white mice by bites of experimentally infected *Tadarida mexicana* and *Antrozous pallidus*, although he found the saliva infectious by intracerebral inoculation. Stamm *et al.* (*3*) also found the saliva of one experimentally infected bat (*Myotis lucifugus*) infectious on intracerebral inoculation. However, what evidence exists for transmission of infection by the bites of insectivorous bats rests upon the occurrence of infection in human beings. At the present time, one well substantiated (*4*) and two possible (*5*) infections have been reported in North America. The present report records infection in white mice resulting from the bite of a naturally infected little brown bat.

The bat (*Myotis californicus californicus*) (*6*) was captured by elk hunters on 21 September 1958 in the Bitterroot Mountains of western Montana, where it attacked one of them twice. The first attack occurred at midday of a sunny day while the hunter was standing in camp. The bat suddenly appeared, lit on the front of his shirt, and bit the fabric. The hunter, a technician in the Rocky Mountain Laboratory, was aware