mixtures consisted of the negatively correlated substances and the insecticides, is essentially different from the usual "Joint toxic action", because the procedure described here is applicable only for the mixed population consisted of the insecticide-resistant and susceptible flies.

Acknowledgments

The author wishes to express his sincere gratitude to Prof. H. Kikkawa for his kind guidance and encouragement. This work was supported by a Research Grant from the W. H. O. and by a Grant for Scientific Research from the Ministry of Education, Japan.

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Genetical Relationship between Ali-Esterase Activity and Insecticide-Resistance in *Drosophila melanogaster*. Genetical and Biochemical Studies on Negatively Correlated Cross-Resistance in *Drosophila melanogaster* IV. Zenichi OGITA (Department of Genetics, Faculty of Medicine, Osaka University, Osaka, Japan). Received July 26, 1961. *Botyu-Kagaku*, 26, 93, 1961 (in English).

16. キイロショウジョウバエにおけるアリエステラーゼ活性と薬剤抵抗性との遺伝学的関係.

キイロショウジョウバエにおける negatively correlated cross-resistance の遺伝生化学的研究 IV. 荻田善一（大阪大学医学部遺伝学教室） 36. 7. 26. 受理.

最近，Van Asperen や Oppenoorth は diazinon や malathion に対して抵抗性の系統のイエバエがもつているアリエステラーゼの活性（methylbutyrate 加水分解活性）が，非抵抗性のイエバエのもつている活性に比して低いことを報告した．この関係がショウジョウバエにおいても存在するかどうかをしらべるために，DDT，BHC，parathion-抵抗性の系統と非抵抗性の系統とを用いてアリエステラーゼとこれらの薬剤抵抗性の関係を遺伝学的に解析した結果，キイロショウジョウバエにおいては DDT, BHC, parathion に対して交叉抵抗性をもたらす第Ⅱ染色体上の gene とは関係なく，アリエステラーゼ活性に関係する遺伝因子は第Ⅲ染色体上に存在することが明らかとなつた．

Recently, Van Asperen and Oppenoorth reported that organophosphorous-resistant strains of housefly which have single gene responsible for the diazinon- and malathion-resistances, exhibited lower ali-esterase activity.

The author found that methylbutyrate was not hydrolyzed by homogenate of two mutants of *D. melanogaster*, while homogenate of wild flies had highly methylbutyrate-splitting capacity. The relation between low ali-esterase activity and insecticide-resistance was studied in the DDT, BHC and parathion-resistant and susceptible strains.

These results obtained led to the conclusion that low ali-esterase activity and DDT, BHC and parathion-resistances are independent on each other, and that the factor responsible for the activity of ali-esterase was involved in the 3rd chromosome.

Introduction

Recently, Van Asperen and Oppenoorth[1,2,3] reported that organophosphorous-resistant strains of housefly exhibited lower ali-esterase activity which seemed to depend upon single gene in both a diazinon-resistant and a malathion-resistant strains.

As it may bring solution on the mechanism of negatively correlated cross-resistance, it appeared to be of some interest to ascertain whether the same phenomenon is recognized in *D. melanogaster* or not. Therefore, genetical analysis of the factors responsible for ali-esterase in this insect has been performed.

Methods and Materials

The following strains of *D. melanogaster* were

used :

bw ; *st ss*, *bw* ; *st* ; *sv*^{n} : multichromosomal mutant strains, susceptible to DDT, BHC, parathion and nicotine sulfate which have been bred for many years in our laboratory.

Canton-S : an unusually DDT-susceptible strain, but moderately resistant to nicotine sulfate.

Hikone-R : resistant not only to DDT, but also to various insecticides such as BHC, parathion, nicotine sulfate etc.

KSL : a highly parathion-resistant strain which was selected by Dr. B. Rasmuson in Sweden. This strain is also highly resistant to DDT and BHC, but moderately resistant to nicotine sulfate.

+ ; + ; HR_3 : a DDT-susceptible, but nicotine sulfate-resistant strain with the 3rd chromosome of the *Hikone-R* strain, and with the 1st and 2nd chromosomes of the *Canton-S* strain.

bw ; HR_3 : a nicotine sulfate resistant strain with the 3rd chromosome derived from the *Hikone-R*, and with the 2nd chromosome of the *bw* ; *st ss* strain.

About two hundred eggs for each culture bottle were reared on the usual medium adding 3% yeast powder. All culture bottles were incubated at 25°C. About one week later pupae were transferred into a glass tube. To synchronize the age, flies emerged at intervals of 12 hours were collected and the flies were kept during 1~2 days for starvation on moistened filter paper in a glass tube.

Unless stated otherwise, ali-esterase activity was assayed by the method as follows : 25 adult flies, 1~2 days old, were homogenized for 30 seconds with a Potter-Elvehjem homogenizer in 4.5 m*l* of 0.15 M NaCl solution under the cold. Esterase activity was usually determined by the Warburg manometric method at 27°C in a total volume of 2.4 m*l* of solution ; 1.8 m*l* of fly homogenates were put in the main compartment, and 0.6 m*l* of substrate solution (100 mM methylbutyrate, 100 mM $NaHCO_3$ in 0.15 M NaCl solution) were pipetted into the side arm. Flasks containing homogenate were kept in ice-water until further use. Cell compartments were filled with gas mixture containing 95 per cent N_2 and 5 per cent CO_2. Then contents of the side arm were tipped in. The temperature equilibration was attained 5 minutes later and then 6 readings were followed at 10 minutes intervals.

1) Ali-esterase activities in various strains and those in their hybrids.

In Fig. 1 and Table 1, ali-esterase activities

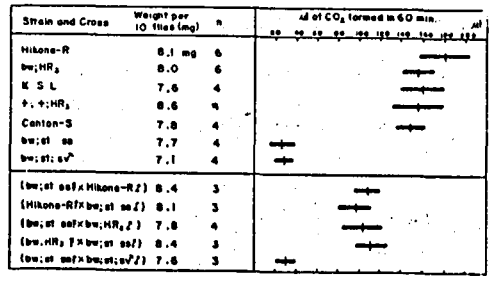

Fig. 1. Ali-esterase activity of male flies in various strains and in their hybrids. (Mean methylbutyrate splitting activity as expressed in μl CO_2/60 min. with its confidence range at 5% level.)

Ali-esterase activities of 1~2 days old 10 male flies of various strains are represented by histgraphs.

Table 1. Ali-esterase activity in female flies of various strains and in their hybrids. (Mean methylbutyrate splitting activity as expressed in μl CO_2/60 min.)

Strain	Weight per 10 flies	*n*	μl of CO_2 formed in 60 min.
Hikone-R	11.2 mg	2	359
bw ; HR_3	11.2 mg	2	303
bw ; *st ss*	10.8 mg	2	58
(*bw* ; *st ss* ♀×*Hikone-R* ♂)	11.2 mg	2	157
(*Hikone-R* ♀×*bw* ; *st ss* ♂)	10.8 mg	2	138
(*bw* ; *st ss* ♀×*bw* ; HR_3 ♂)	11.2 mg	2	162
(*bw* ; HR_3 ♀×*bw* ; *st ss* ♂)	11.2 mg	2	168

of male and female in various strains and in their hybrids were expressed as μl of CO_2 formed in 60 minutes in manometer compartments.

High activity is found in *Hikone-R*, *KSL*, *Canton-S*, *bw* ; HR_3 and + ; + ; HR_3 strains, while low activity in *bw* ; *st ss* and *bw* ; *st* ; sv^n strains. These results indicate that ali-esterase activity of *D. melanogaster* has no correlation with DDT-, BHC- and parathion-resistance.

It became also clear that ali-esterase activity of female was higher than that of male, and that the activities of their hybrids between low activity strain, and high activity strains were intermediate to those of both parent strains. Moreover the maternal or cytoplasmic effects were almost negligible.

Ali-esterase activity of F_1 hybrid obtained from the cross *bw* ;*st ss*♀ ×*bw* ; *st* ; sv^n♂ was found to have no difference from low activity of both parent strains. Therefore, it may be assumed that the genes involved in these two mutant strains are identical with each other for low activity.

2) Change of ali-esterase activity with age of adult flies.

As shown in Fig. 2, it was indicated that the levels of ali-esterase activities were varied with the age of flies in condition of starvation with moistened filter paper ; that is, ali-esterase activity of 1～2 days old fly was the highest. Therefore, it is necessary to compare the levels of activities of both parent flies with those of their hybrids by using 1～2 days old flies obtained from synchronized cultures.

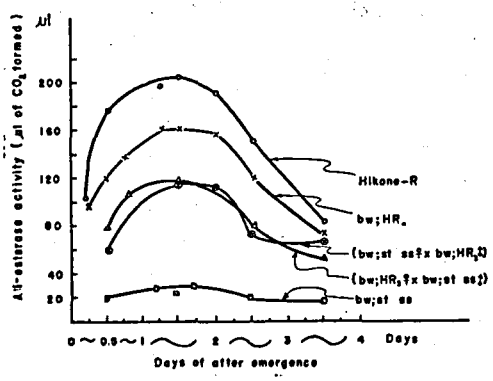

Fig. 2. Change of ali-esterase activity with age of adult flies.
Ali-esterase activities of 10 male flies in various ages represented by points, indicating the amount of CO_2 in 60 minutes. The days show a period of starvation in a glass tube with moistened filter paper.

3) Chromosomal location of factor responsible for the ali-esterase activity.

In order to determine chromosome which is responsible for ali-esterase activity, the relation between the phenotypes and the esterase activities in F_2-progenies obtained from the backcross *bw*; *st ss*♀ ×F_1(*bw* ; *st ss* ♀ ×*Hikone-R*♂)♂ was examined by the manometric method.

As shown in Fig. 3, the activity of each phenotypical F_2-progenies obtained from the backcross showed a clear segregation ; ali-esterase activities of *bw* ; ++ and + ; ++ (wild type) which had the 3rd chromosome carrying the factor of high activity derived from *Hikone-R* in heterozygous condition, were higher than those of *bw* ; *st ss* and + ; *st ss* progenies which had the 3rd chromosome carrying the low activity derived from *bw* ; *st ss* in homozygous condition.

These data evidently indicate that the recessive gene or genes for the low activity are linked with the *st* and *ss* characters, *i.e.*, with the 3rd chromosome.

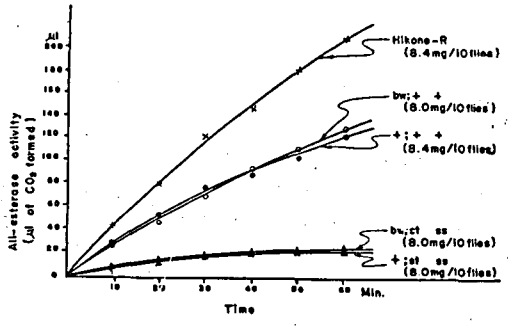

Fig. 3. Ali-esterase activity of male of each phenotypical F_2-progeny obtained from the backcross *bw*; *st ss*♀ ×F_1(*bw*; *st ss*♀ ×*Hikone-R*♂)♂.
The 1～2 days old 10 male flies after emergence were used to determine ali-esterase activities of F_2-progenies. The *bw* ; ++ and + ; ++ (wild type) progenies have the 3rd chromosome derived from *Hikone-R* respectively in heterozygous condition. The *bw* ; *st ss* and + ; *st ss* progenies have the 3rd chromosomes derived from *bw* ; *st ss* respectively in homozygous condition.

Table 2. Distribution of ali-esterase and cholinesterase activities in the tissue of *D. melanogaster*.

Strain	Weight of tissue from 25 flies		Ali-esterase activity	Cholinesterase activity
Hikone-R ♂	Head	1.7 mg	26.0 μl CO_2 9.7%	80.0 μl CO_2 48.7%
	Body*	14.5 mg	242.5 μl CO_2 90.3%	84.3 μl CO_2 51.3%
bw ; *st ss* ♂	Head	1.6 mg	2.0 μl CO_2 4.0%	84.6 μl CO_2 47.6%
	Body*	14.7 mg	48.6 μl CO_2 96.0%	93.0 μl CO_2 52.4%

* Body=thorax+abdomen.

The data in the table were obtained with the Warburg manometric method, by using homogenate with 25 flies. Ali-esterase activity was expressed as capacity of splitting methylbutyrate. Cholinesterase activity was expressed as capacity of splitting acetylcholine.

4) Localization of ali-esterase and cholinesterase.

The 50 flies were decapitated, and the heads and the rest of the bodies were separately homogenized in 2 m*l* of 0.15 M NaCl solution in the cold. Activities of ali-esterase and of cholinesterase in 1 m*l* of homogenates of the heads and of bodies (which contained the thoraces, abdomens and all the appendages) were compared with each other. The results are shown in Table 2.

These data indicate that the distribution of ali-esterase in the body was distinctly different from that of cholinesterase, and that cholinesterase activity shows no consistent difference between the high ali-esterase and low ali-esterase strains.

Discussion

Van Asperen[2] worked on hydrolytic activities in homogenates of houseflies by using several substrates. It has been suggested that hydrolytic activities on different substrates used could be explained by the action of two main enzymes ; one is cholinesterase, and the other is ali-esterase which is defined as the enzyme capable of splitting methylbutyrate. Therefore, these enzyme activities in various strains of *D. melanogaster* were determined by the Warburg manometric method with methylbutyrate and acetylcholine-chloride, as substrates.

From the data given in Fig. 1 and Table 1 on ali-esterase activities of various strains and of their F_1-hybrids which were obtained from reciprocal crosses, it became clear that low ali-esterase activity was controlled by the autosomal recessive gene or genes which had no correlation with the DDT-, BHC- and parathion-resistant factors.

Methylbutyrate was not hydrolyzed by homogenates of two mutants of *D. melanogaster* (*bw* ; *st ss* and *bw* ; *st* ; *sv*^{n} strains), while homogenate of wild flies had highly methylbutyrate-splitting capacity. The lack of methylbutyrate-splitting capacity in these mutant strains may be due to the absence of the gene or genes which control this specific enzyme formation, or due to the presence of the gene or genes which control the specific inhibitor formation for this enzyme.

The results of analyses of F_2-progenies obtained from the backcross *bw* ; *st ss*♀ × F_1(*bw* ; *st ss*♀ × *Hikone-R* ♂)♂, indicated that the low activity was associated with the *st* and *ss* characters, *i.e.* with the 3rd chromosome (Fig. 3). Although it is still premature to conclude that the low activity is controlled by only one gene, it is called *ali* for convenience' sake. Therefore, its allele with high activity may be represented by the symbol *ali*$^+$.

It is very interesting that the activity of F_1-hybrid (*ali*/*ali*$^+$) obtained from the crosses of low activity flies (*ali*/*ali*) and high activity flies (*ali*$^+$/*ali*$^+$) reveals an intermediate level of the parent strains. This result is similar to that reported in housefly by Oppenoorth[3]. As shown in Fig. 2, levels of ali-esterase activities of these flies were varied with the age of fly. Therefore, only in the synchronized culture condition such as used in this experiment, the three groups, *ali*/*ali*, *ali*/*ali*$^+$ and *ali*$^+$/*ali*$^+$, could be distinguished in the level of enzyme activity.

As shown in Table 2, the cholinesterase activity of a low ali-esterase *bw*; *st ss* strain and of a high ali-esterase *Hikone-R* strain, showed no difference. And also, the distribution of ali-esterase was found to be distinctly different from that of cholinesterase. That is, ali-esterase activity was mainly found in the body and slightly in the head, but cholinesterase activity was not so. These results suggest that the two esterase activities may be controlled by different genes.

Further research is now in progress on ali-esterase from the stand point of biochemical genetics.

Acknowledgments

The author wishes to express his sincere gratitude to Prof. H. Kikkawa for his kind guidance and encouragement. This work was supported by a Research Grant from the W. H. O. and by a Grant for Scientific Research from the Ministry of Education, Japan.

Summary

1) Genetical analyses on factor responsible for the low activity of ali-esterase suggested that the factor was involved in the 3rd chromosome, and that it was not correlated with the DDT-, BHC- and parathion-resistant factors.

2) Ali-esterase activity of F_1-hybrids obtained from the reciprocal crosses between flies of low activity and those of high activity showed an intermediate level of the parent strains.

3) Cholinesterase activities, in strains of low and high ali-esterase activities, were quite similar to each other. And also the distribution of cholinesterase in parts of the body was distinctly different from that of ali-esterase. The results suggest that these esterase activities are controlled by different genes.

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抄 録

蚕蛾の性誘引物質 *Bombykol* の全幾何異性体の合成

A. Butenandt und E. Hecker, Synthese des *Bombykols*, des Sexual-Lockstoffes des Seidenspinners, und seiner geometrischen Isomeren. *Angew. Chem.*, **11**, 349 (1961)

先に雌蛾50万頭より抽出した性誘引物質は Hexadekadien-(10,12)-ol-(1) で，その幾何構造は 10-*cis*, 12-*trans* か 10-*trans*, 12-*cis* のいずれかであることを明かにしたが，今回その4異性体全部を合成し，天然物は 10-*trans*, 12-*cis* 体であることを明かにした．

I) C_7+C_9; Propylbromid と *n*-Butyraldehyd から *Reformasky* 反応で 4-Hydroxy-heptin をえ，このものの Tosylat を 30%KOH 溶液で処理すると，*cis*-Hepten-3-in-(1)(70%) と *trans*-(30%) の混合体をえる．このものは高性能の分溜管で分溜をくり返し *cis*, *trans* を分別する．[1] 次いで C_9 化合物として Nonamethylenglykol を HBr で ω-Brom-nonanol-(1) とし，これを Tetrahydropyran で OH 基を保護する．この Pyranyläther を先に合成した C_7 化合物 Hepten-3-in-(1) の各々 *cis* 体と *trans* 体と反応させてから Äthergruppe をはずすと Hexadecin-10-en-12-ol-(1) の *cis*, *trans* 混合体をえる．これを高真空蒸溜をくり返し，更に誘導体をへて精製単離する．[1] このものを *Lindlar* 触媒で半水添後誘導体として精製単離する．このようにしてえた 10-*cis*, 12-*cis* II および 10-*cis*, 12-*trans* III のうち後者の IR は天然物のそれと少し異るが，[2] その 4'-Nitro-azobenzol-carbonsäure-4-ester は混融降下を示さない．10-*cis*, 12-*trans* 体を I_2 の存在下異性化させて 10-*trans*, 12-*trans* V をえた．[3]

II) C_6+C_{10}; Pentin-(1) の Grignard 化合物に Formalin ガスを作用させてえた，Hexin-2-ol-(1) を PBr_3 で Bromid とする．これを Triphenylphosphin で Phosphoniumbromid ($Br^{\ominus\oplus}[R_3\text{-}P\text{-}CH_2\text{-}C\equiv C\text{-}C_3H_7]$),

1) このものは各々の異性体がごく微量混在していることが IR によつて認められる．

2) Alkohol基の面外伸縮振動の位置が少しずれる．

3) 10-*cis*, 12-*cis*: schmp, 25.5～26.5°, 10-*trans*, 12-*trans*: schmp 27～38°, 他の2異性体は液体．