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Strain Differences in Minimum Anesthetic Concentrations in Drosophila melanogaster

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An ether-resistant strain of *Drosophila melanogaster* has been maintained at this laboratory since the appearance of one female mutant in 1961. Sensitivity was defined using mortality as an endpoint when exposed to a high concentration of diethylether; this does not necessarily mean that anesthetic requirements are higher in the resistant strain. The present study was undertaken to determine the difference in anesthetic potency between the ether-resistant strain (*Eth*-29) and one of the sensitive strains (*bw;st;sv*^{n}). The median effective dose (ED_{50}) for halothane was 0.0096 atm in females and 0.0091 atm in males of the *bw;st;sv*^{n} strain, while in the *Eth*-29 strain the ED_{50} was 0.0148 atm in both sexes. The ED_{50} values for chloroform anesthesia were 0.0051 atm in females and 0.0050 atm in males of the *bw;st;sv*^{n} strain and 0.0100 atm in the *Eth*-29 strain in both sexes. Strain differences in response to the two anesthetics were statistically significant. Thus the *Eth*-29 strain shows a cross-resistance to both halothane and chloroform anesthesia. Reciprocal crosses between the two strains revealed that the resistance to halothane anesthesia was a sex-linked recessive trait and that the resistance to chloroform anesthesia was an autosomal incompletely dominant trait. (Key words: Anesthetics, volatile: halothane; chloroform. Potency, anesthetic: ED_{50}; genetics.)

IN 1961 a spontaneous ether-resistant female fly appeared in our laboratory. The offspring of this mutant fly were carefully screened for ether-resistance and were named *Eth* strain. For controls, more than one hundred stock strains were examined for their ether-sensitivity and the more ether-sensitive strains were used. Genetical studies of resistance to the lethal effects of volatile anesthetics have been carried out using these resistant and sensitive strains. The *Eth* strain was found to be resistant to ether[1], chloroform[2], and halothane[3] when compared with the control strains. The genetic character of both resistances to ether and to chloroform is completely dominant. The major gene for ether-resistance is located on the third chromosome, while that for the chloroform is located on the first (X) chromosome. Minor genes for ether-resistance are also different from those for chloroform-resistance. These observations indicate that resistance to ether and to chloroform is controlled by different genes. The resistance to halothane is an incompletely dominant trait, this major gene is located on the third chromosome and the minor genes are located on the X and second chromosomes. Thus, although halothane and chloroform are both halogenated hydrocarbons, the genetic basis of the resistance to halothane differs from that to chloroform. Since the lethal effects of anesthetic agents need not reflect anesthetic potency, the present study was carried out to determine the anesthetic requirements of the resistant and sensitive strains.

Materials and Methods

The ether-resistant strain (*Eth*-29) and one of the sensitive strains (*bw;st;sv*^{n}), which were previously established in the studies of anesthetic-induced mortality,[1–3] were used for the determination of median effective dose (ED_{50}) for halothane and chloroform. The *Eth*-29 strain was procured by the selection of the *Eth* flies resistant to deep ether anesthesia for 29 generations so as to purify the genetic background of the original *Eth* strain. The *bw;st;sv*^{n} strain is a multichromosomal recessive mutant which carries each gene for the brown eye, scarlet eye, and shaven-naked bristles on the second, third, and fourth chromosome. In order to investigate the genetic basis of strain differences in halothane and chloroform requirements, reciprocal crosses between the two strains wee carried out (fig. 1).

The flies were cultured on a larval medium (15 per cent crude sugar, 2 per cent agar, 5 per cent rice-bran, and 2 per cent dry yeast in water) at 25 ± 0.5° C, as previously described.[1] For each trial, 30 one-day-old virgin flies of either sex were used as one group, and were anesthetized for 30 min in a filtering flask (200 ml) with varying concentrations of the anesthetics. Over-flow gas was vented from the flask through a glass tube inserted into the silicon rubber stopper of the flask. The stopper was removed only when the flies were transferred into and out of the flask. During the first 20 min, the number of anesthetized flies gradually increased and reached a steady-state, after which no consistent rise or fall occurred from 20 to 40 min. We therefore kept flies for 30 min at a given concentration of an anesthetic agent prior to the determination

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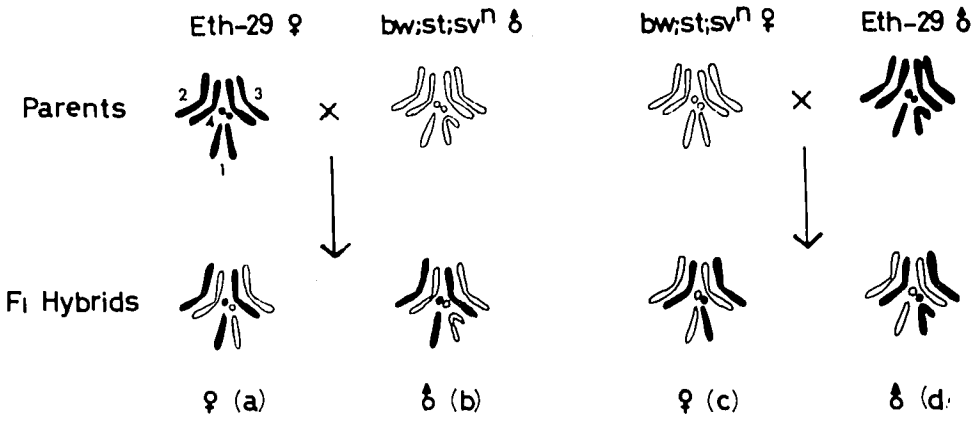

FIG. 1. Chromosome constitution in the *Eth*-29 and *bw;st;sv*^{n} strains and their F_1 hybrids. The constitution of autosomal chromosomes (2nd 3rd and 4th) is the same in all F_1 progenies. The sex chromosomes in *Drosophila melanogaster* are XX in female and XY in male. Sex differences in either cross indicate sex linked character. Difference between F_1 females (a) and (c) indicates presence of a maternal effect.

of the anesthetic effects. Then, the flies were quickly removed from the anesthetizing chamber and placed on a white plate (7 × 15 cm) under a binocular microscope (10X, Nikon®), and stimulated 5 times by a small paint brush. Limb movement in response to the stimulus was the observed endpoint. The period of observation was usually 2–3 min. The number of flies not responding was recorded. We then transferred the flies to a fresh medium; recovery of normal flying activity was usually complete within 30 min. At the tested concentrations, neither anesthetic had apparent toxic effects on the flies.

Halothane and chloroform were vaporized in a Copper Kettle® and diluted with oxygen (2 l/min). The concentration of the anesthetic gas was estimated from Copper Kettle® temperature and the flow of diluent oxygen. In addition, the concentration of anesthetic gases was checked by gas chromatography. The room temperature was controlled at 25 ± 1.0° C throughout the experiments. We used reagent grade chloroform and clinical grade halothane. All anesthetics were protected from the light and stored in a refrigerator.

TABLE 1. Halothane and Chloroform ED_{50} Values with SE in the *Eth*-29 and *bw;st;sv*^{n} Strains and Their F_1 Hybrids of Reciprocal Crosses Between the Strains

Strain or Hybrid	Sex	Halothane (atm)	Chloroform (atm)
bw;st;sv^n	♀	0.0096 ± 0.00080	0.0051 ± 0.00057
	♂	0.0091 ± 0.00090	0.0050 ± 0.00048
Eth-29	♀	0.0148 ± 0.00060	0.0100 ± 0.00040
	♂	0.0148 ± 0.00066	0.0100 ± 0.00042
bw;st;sv^n/*Eth*-29*	♀	0.0101 ± 0.00078	0.0061 ± 0.00078
	♂	0.0097 ± 0.00088	0.0058 ± 0.00072
Eth-29/*bw;st;sv*^{n}†	♀	0.0116 ± 0.00075	0.0077 ± 0.00065
	♂	0.0136 ± 0.00076	0.0078 ± 0.00068

* *bw;st;sv*^{n} females and *Eth*-29 males.
† *Eth*-29 females and *bw;st;sv*^{n} males.

Quantal responses at each anesthetic dose were used to construct a dose-response curve for each strain and F_1 hybrid. Logarithm-probit transformation was employed for the linearization of the dose-response curve, and ED_{50} with standard error was determined. We estimated a common slope for the dose-response curves to compare them and analysis of covariance was carried out to examine fitness of lines. Differences in response among regression lines with a common slope were tested by multiple *t* test. All calculations were carried out with a computer system (NTIS ACOS Series 77 Model 600®).

Results

Linear regression equations for dose-response curves were calculated to obtain ED_{50} in *bw;st;sv*^{n}

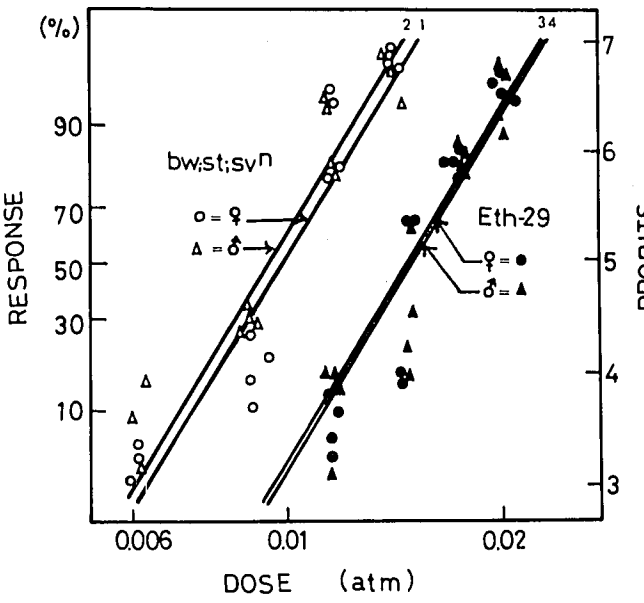

FIG. 2. Regression lines with a common slope for halothane in parent strains: line 1 = *bw;st;sv*^{n} females; line 2 = *bw;st;sv*^{n} males; line 3 = *Eth*-29 females; and line 4 = *Eth*-29 males.

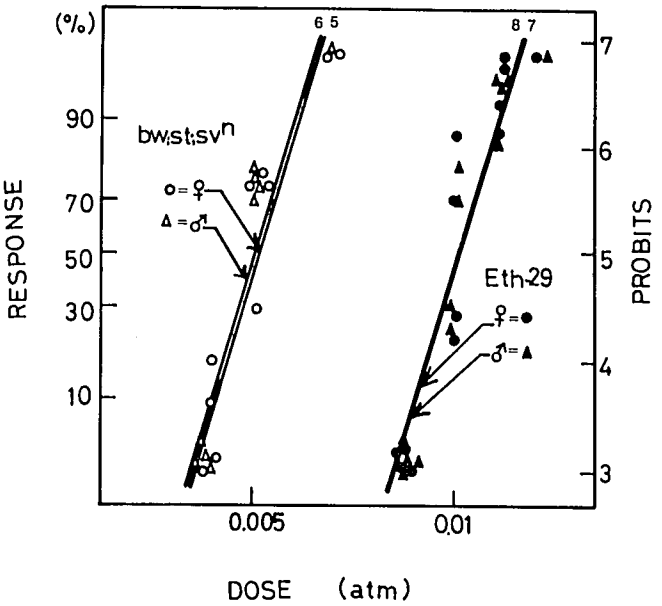

FIG. 3. Regression lines with a common slope for chloroform in parent strains: line 5 = *bw;st;sv*^{n} females; line 6 = *bw;st;sv*^{n} males; line 7 = *Eth*-29 females; and line 8 = *Eth*-29 males.

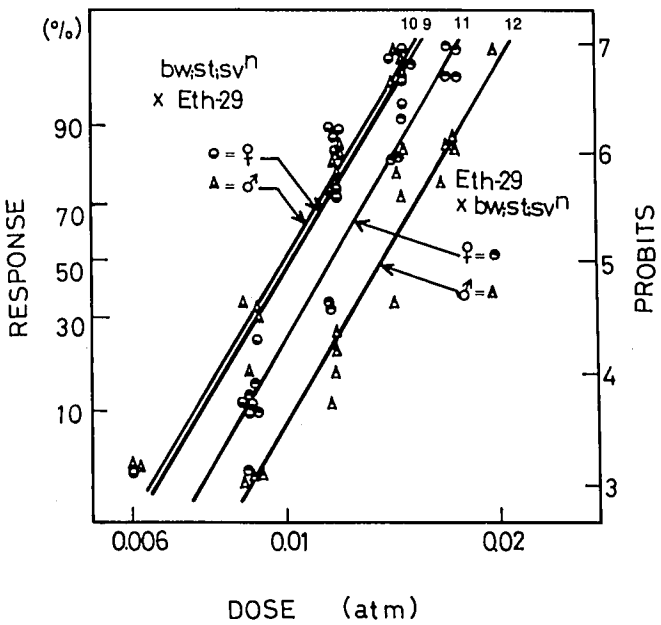

FIG. 4. Regression lines with a common slope for halothane in F_1 hybrids: line 9 = *bw;st;sv*^{n}/*Eth*-29 females; line 10 = *bw;st;sv*^{n}/*Eth*-29 males; line 11 = *Eth*-29/*bw;st;sv*^{n} females; and line 12 = *Eth*-29/*bw;st;sv*^{n} males.

and *Eth*-29 strains and their F_1 hybrids. The ED_{50} values with standard errors are presented for halothane and for chloroform (table 1). Regression lines with a common slope for *bw;st;sv*^{n} females, *bw;st;sv*^{n} males, *Eth*-29 females and *Eth*-29 males are shown in figure 2 for halothane, and in figure 3 for chloroform. From the analysis of covariance, a common slope was acceptable. Differences among regression lines with a common slope were tested by multiple *t* tests (table 2*A*). Although strain differences were significant for halothane and for chloroform, sex differences were not significant in either of the strains.

In F_1 progenies from reciprocal crosses between the two strains, the analysis of covariance also showed that a common slope was fit to the regression lines of F_1 hybrids (fig. 4 and 5). The multiple *t* tests for these lines are presented in table 2*A*.

Eight parallel lines with a common slope were tested by multiple *t* test to compare F_1 hybrids with their parent strains (table 2*B*) and to examine the genetical character of the resistance to halothane anesthesia and that to chloroform anesthesia.

Discussion

In the previous studies[1-3] examining comparative lethality of anesthetics in *Drosophila melanogaster*, the *Eth*-29 strain was more resistant to ether,[1] chloroform,[2] and halothane[3] than the ether-sensitive strains. In this study, strain differences in sensitivity for halothane and chloroform anesthesia were established. The *Eth*-29 strain was resistant to both halothane and chloroform anesthesia when compared with the *bw;st;sv*^{n} strain, and sex differences were not found in either of the strains (fig. 2 and 3).

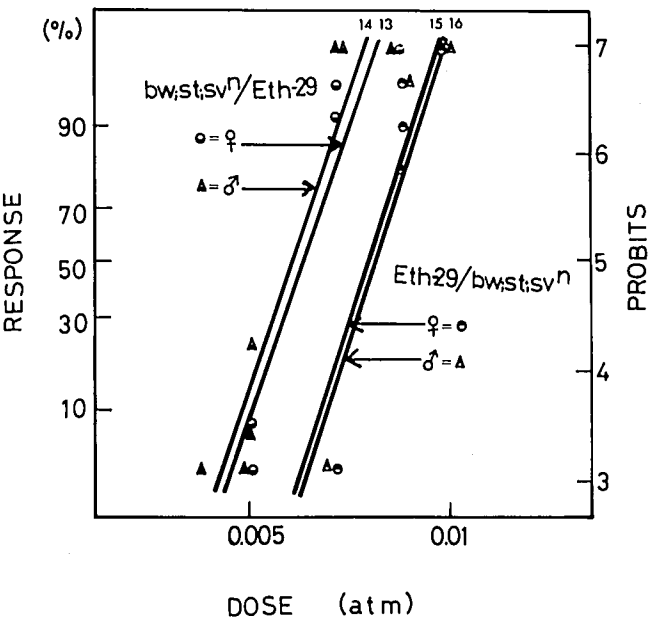

FIG. 5. Regression lines with a common slope for chloroform in F_1 hybrids: line 13 = *bw;st;sv*^{n}/*Eth*-29 females; line 14 = *bw;st;sv*^{n}/*Eth*-29 males; line 15 = *Eth*-29/*bw;st;sv*^{n} females; and line 16 = *Eth*-29/*bw;st;sv*^{n} males.

TABLE 2. Multiple *t* Test Among Dose-Response Curves in the *Eth*-29 and *bw;st;sv*^{n} Strains and Their Hybrids

Strain or Hybrid*	Halothane	Chloroform
(*A*) (1) *vs.* (2)	0.602	0.328
(3) *vs.* (4)	0.103	0.020
(1) *vs.* (3)	5.146§	7.709§
(2) *vs.* (4)	5.668§	7.557§
(5) *vs.* (6)	0.424	0.455
(7) *vs.* (8)	2.999‡	0.143
(5) *vs.* (7)	2.349†	1.957
(6) *vs.* (8)	5.509§	2.442†
(*B*) (5) *vs.* (1)	0.486	1.350
(5) *vs.* (3)	3.794§	3.332§
(6) *vs.* (2)	0.693	1.252
(6) *vs.* (4)	4.145§	3.672§
(7) *vs.* (1)	2.004†	2.475†
(7) *vs.* (3)	2.601‡	1.981
(8) *vs.* (2)	4.229§	2.647‡
(8) *vs.* (4)	0.860	2.041

* Regression lines with a common slope: (1) *bw;st;sv*^{n} ♀, (2) *bw;st;sv*^{n} ♂, (3) *Eth*-29 ♀, (4) *Eth*-29 ♂, (5) *bw;st;sv*^{n}/*Eth*-29 ♀, (6) *bw;st;sv*^{n}/*Eth*-29 ♂, (7) *Eth*-29/*bw;st;sv*^{n} ♀, and (8) *Eth*-29/*bw;st;sv*^{n} ♂.
† $P < 0.05$, ‡ $P < 0.01$, and § $P < 0.001$.

A comparison of ED_{50} values in a variety of species is given in table 3. The ED_{50} values for both halothane and chloroform in *bw;st;sv*^{n} strain are close to those of other species, while those of the *Eth*-29 strain are higher. Thus the *Eth* strain which was originally identified as a spontaneous ether-resistant mutant, is also a resistant mutant for halothane and chloroform anesthesia. The differences in anesthetic requirements which amount to 50–100 per cent between the two strains, are much larger than those found due to tolerance[17,18] or circadian rhythms[19] (10–20 per cent), but are rather similar to the differences due to a 10° C alteration in body temperature.[6,13,20,21] Therefore, we postulate that the differences between the *Eth*-29 and *bw;st;sv*^{n} strains may be related to changes of lipid (or protein) composition in cell membranes. However, Koblin, *et al.*[22] reported no significant difference in synaptic membrane lipid composition (phospholipid, fatty acid and cholesterol) in selectively bred mice either resistant to or susceptible to nitrous oxide anesthesia, although genetical differences in the two groups of mice were not proved.

Sex differences in F_1 hybrids of one of the reciprocal crosses for halothane anesthesia indicates that the resistance to halothane anesthesia in the *Eth*-29 strain is a sex-linked character. Few sex differences in F_1 hybrids of both crosses for chloroform anesthesia were found, indicating that the resistance to chloroform anesthesia is an autosomal character. The differences in sensitivity for both halothane anesthesia (fig. 4) and chloroform anesthesia (fig. 5) between F_1 females of both crosses indicate the presence of a maternal effect. But no maternal effect on resistances to lethality of anesthetics was found previously.[1–3]

In order to examine dominancy of each resistance, F_1 hybrids were compared with their parent strain (table 2*B*). The lines of *bw;st;sv*^{n}/*Eth*-29 for halothane were close to the *bw;st;sv*^{n} strains and those of F_1 hybrids of both crosses for chloroform were intermediate between their parents. From these findings, we conclude that the resistance to halothane anesthesia is a recessive sex-linked character and the resistance to chloroform anesthesia is an incompletely dominant autosomal character. Thus the genetical basis for the resistance to halothane and chloroform anesthesia differs. Presumably these two resistances are controlled by different genes. This means that the mechanism for each resistance may not be the same.

TABLE 3. Comparison of Different Estimates of Anesthetic Potency (ED_{50} or MAC)

Species	Halothane (atm)	References	Chloroform (atm)	References
Drosophila (25° C)				
bw;st;sv^n				
(F)	0.0096		0.0051	
(M)	0.0091		0.0050	
Eth-29				
(F)	0.0148		0.0100	
(M)	0.0148		0.0100	
Luminous bacteria	0.0076–0.0081	4, 5	0.0066–0.0070	4, 5
Firefly (21.5° C)	0.0104	6	0.0090	6
Dog	0.0087	7	0.0077	14
Human	0.0074–0.0077	8, 9		
Mouse	0.0086	10	0.008	15, 16
Monkey	0.010–0.012	10		
Cat	0.0082	11		
Goldfish (25° C)	0.0076	12	0.0063	12
Toad (20–22° C)	0.0068	13		

Generally, the *Eth* strain shows a cross-resistance to the lethality of ether, halothane and chloroform and to both halothane and chloroform anesthesia, though the methods used for determining lethality and anesthesia sensitivity are different. However there is a different genetical basis for each resistant trait of the *Eth* strain. This different genetical basis should play a role in the mechanism for each resistance.

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