Studies on the Biological Assay of Insecticides. (XXXVII)*

On the Differential Susceptibility of the Sexes of the Common House Fly, *Musca domestica vicina* Macquardt, to the Knockdown Effect of *p,p'*-DDT Powder

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Some morphological characters for easily and unmistakably discriminating the sexes of living individuals of the common house fly were described. In order to calculate the degree of difference between the female and the male insects in susceptibility to the knockdown effect of *p,p'*-DDT powder, experiments were carried out using the settling dust apparatus. On the basis of results of experiments, it was emphasized that the numbers of the males and the females used for a test should be as nearly equal as possible when the biological assay is carried out utilizing test insects, the sexes of which are not identified beforehand.

INTRODUCTION

It has been reported by Bruce and Decker[4], Busvine[5], Eagleson[7], Matsubara[15], Miller and Simanton[16], Murray[17], Nagasawa and Uruha[19], Nagasawa,[21,22,23,24] etc. that the female and the male of the common house fly, *Musca domestica vicina* Macq., show considerable differences in response to the stimuli of various toxicants. They pointed out that the males are generally more susceptible to toxicants than the females. When the difference in susceptibility between the sexes is remarkable, it is difficult to arrive at a clearcut dosage-response relation from the experiment in cases where individuals, whose sexes are not identified beforehand, are used. Therefore, it is important to know the degree of this difference. In the present paper, the writer tried first, to show the morphological characters which can be relied upon for easily and unmistakably, discriminating the sexes of living individuals of the common house fly and then, to describe the degree of sexual difference in susceptibility to the knockdown effect of *p,p'*-DDT powder when the settling dust apparatus method is resorted to.

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* This is a rewriting in English prepared from the data in the writer's papers which were published in *Kagaku,* Vol. 21, pp. 591-592(1951), and *Botyu-Kagaku,* Vol. 17, pp. 123-133(1952) and in his monograph "Studies on the Biological Assay of Insecticides, with Special Reference to the Studies of Factors Affecting the Experimental Results of the Settling Dust Apparatus Method" pp. 22-26(1954).

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MATERIAL AND INSECT

***p,p'*-DDT powder** : For the present study, four grades of *p,p'*-DDT powder in the concentration of 0.5, 1.0, 2.0 and 4.0 % were prepared by the solvent application method. The carrier used for preparation of the powder was the pyrophyllite which was quarried at Glendon, N. C., and manufactured to the insecticide grade pyrophyllite by the Carolina Pyrophyllite Co., U. S. A. The method of preparation of the experimental material is as follows : *p, p'*-DDT (mp 107∼108°C) was dissolved in benzol in advance ; the solution was mixed with the carrier in a ratio so as to give a prescribed *p,p'*-DDT content in the resulting dust mixture ; then, the solvent was removed by evaporation at room temperature and the mixture of *p,p'*-DDT and carrier was ground up in a mortar to fine powder. It was screened through the 325 mesh screen of Tyler's standard sieve. The distribution of particles in size was not determined.

The common house fly : The common house flies, *Musca domestica vicina* Macq., used in this experiment were the flies reared under the condition of ca 30°C and ca 50% relative humidity. Paste of wheat powder was used as the food for adult insect and the horse manure was used as the culture medium for larval stage[19,20]; this is a simplification of the Grady's method[9]. The healthy individuals of uniform size 4∼5 days old after emergence were used for experiment. This house fly was the progeny of the flies which have been reared for many years in the writer's laboratory by inbreeding a small number of normal individuals Therefore these individuals are considered to be a pure strain having the same genetical structure so that it may be expected that they are quite homogeneous in physiological as well as in morphological characters. The writer calls this strain the "Takatsuki strain".

MORPHOLOGICAL DISTINCTION OF SEXES IN LIVING ADULT INSECTS

As the common house fly has no distinct sexual differences in its external morphological characters in the other stages than in the adult stage, it is impossible to discriminate two sexes in eggs, larvae and pupae in advance, to use the adults of the desired sex emerged from them for experiment. The difference in size between the adult females and males can not be recognized in the larval or in the pupal stage. To take an example, the figures in Table 1 are the measurements of length and width of the pupae which were obtained from a single culture medium. The sexes of the pupae were determined after the adults emerged from them. Though the mean length and width of the female pupae seems to be a little larger than that of the male pupae, statistically there were no significant differences either in the mean length or in the width between the female and the male pupae at the 5 per cent level. That is, $F_0=0.92<F^1_{211}(0.05)$ for length and $F_0=0.73<F^1_{211}(0.05)$ for width.

Table 1. Measurements on the length and width of female and male pupae of the common house fly, *Musca, domestica vicina* Macq. 1 unit=0.098mm.

Sex	Number of individuals	Dimension	Mean	Standard deviation	Coefficient of variation
Female	132	Length	72.371±0.248	±2.846	3.93%
		Width	30.008±0.129	±1.480	4.93
Male	81	Length	71.221±0.264	±2.373	3.33
		Width	29.469±0.142	±1.277	4.33

For the reason mentioned above, when it is necessary to carry out experiments using individuals of which the sexes are known, we must immobilize the test insects in advance by low temperature or by some anaesthetics and group them into the female and the male insects while they are in immobile state. And also, when the influences of low temperature or anaesthetics must be taken into consideration, it is necessary to group them into two sexes by taking out a portion of the stock flies at a time from the stock cage by means of small glass tubes. Because of these circumstances, it is desirable to discriminate sexes by some external morphological characters. In the adult stage, we can easily and unmistakably determine the sexes of living individuals by the following three characters.

(1) Ratio of frons to width of head. As shown in Figs. 1 and 2, the ratio of frons to width of head in the male is larger than that in the female. This character is fairly common to many dipterous insects. In the common house fly, this character is visible both in the frontal and back aspects. The results of measurments are shown in Table 2. Needless to test the significant difference between the means

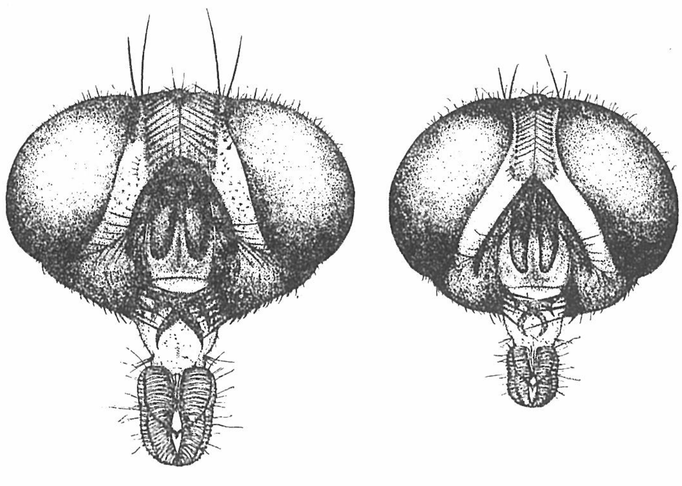

Fig. 1. Frontal aspect of heads of the common house fly, *Musca domestica vicina* Macq. Left, female ; right, male.

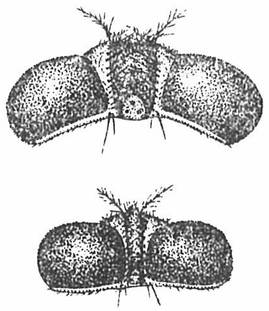

Fig. 2. Dorsal aspect of heads of the common house fly, *Musca domestca vicina* Macq. Above, female ; below, male.

Table 2. Sexual difference in the ratio of width of frons to width of head of the common house fly, *Musca domestica vicina* Macq.

Sex	Number of individuals	Mean	Standard deviation	Coefficient of variation
Female	330	2.995±0.009	±0.169	5.73%
Male	384	7.848±0.663	±1.300	16.56

statistically, since the significance between the sexes is remarkably large.

(2) Pattern of abdominal tergites. As shown in Fig. 3, in the female, the first tergite is black. The second tergite is generally greyish yellow and has a wide dark brownish median stripe which widens out anteriorly and posteriorly. The third, fourth and fifth tergites are greyish yellow. On the third and fourth tergites, there are a dark brownish median stripe and admedian stripes, the latter spreading out towards the hind border of tergite. The median and admedian stripes on the fifth

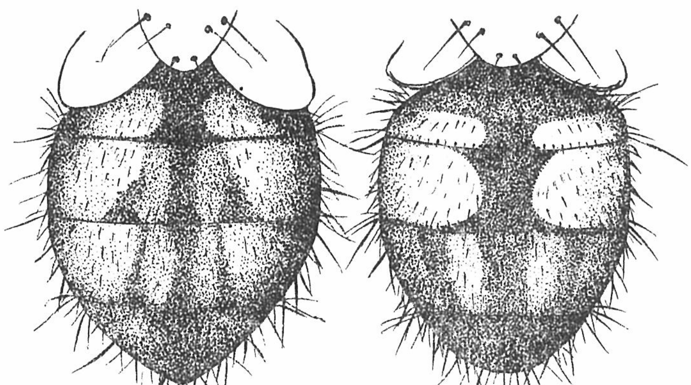

Fig. 3. Dorsal aspect of abdomens of the common house fly, *Musca domestica vicina* Macq. Left, female ; right, male.

tergite are somewhat indistinct. In the male, the first tergite is black. The second tergite is greyish yellow and has a broad brownish median stripe which widens out anteriorly and posteriorly. In the third tergite there is a similar median stripe and the color of the greyish yellow pattern is relatively distinct. The fourth and fifth tergites are greyish, and there are greyish black median and admedian stripes which are rather indistinct in the fifth tergite. This sexual difference, however, is somewhat difficult to recognize in the posture of repose as the wings are folded on the abdomen.

(3) Color and shape of abdominal sternites. As shown in Fig. 4, the end portion of abdominal sternites in the male is roundish and blackish. But in the female, this portion is somewhat pointed and generally whitish except the portion where the ovipositor is extruded. This sexual character is very easily and clearly seen in the dorsal aspect when they perch on the wall of a glass jar or in the state of knockdown.

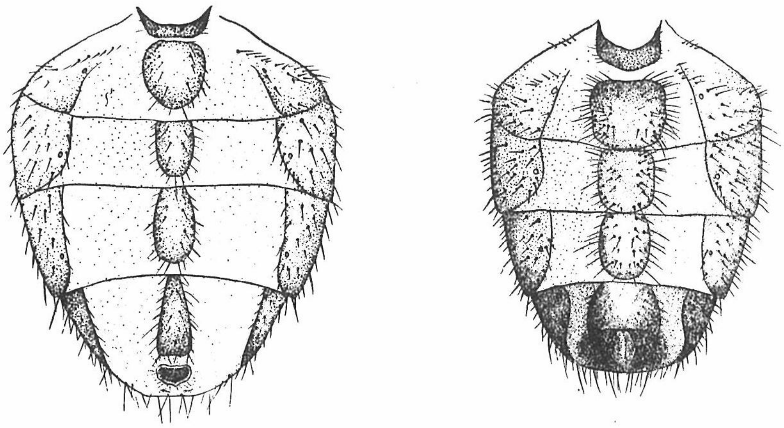

Fig. 4. Ventral aspect of abdomens of the common house fly, *Musca domestica vicina* Macq. Left, female ; right, male.

Besides, the female is usually somewhat larger than the male. When the flies are slightly narcotized with vapour of ether, the majority of females extrude their yellowish white ovipositor from the end of abdomen. But these two characters are not always observable in all the females, so that they are merely supplementary to the other characters for discriminating the sexes in the mature stage.

APPARATUS AND METHOD

The settling dust apparatus method[24] was adopted in the present experiment. As shown in the right of Fig. 5, the settling dust apparatus consists of a glass cylinder (A) 20 cm in diameter and 43 cm in height, and two glass plates (B, C) 27 cm in diameter covering its upper and lower openings. And the glass cylinder is placed on the wooden stand (F) 30 cm in height. About twenty healthy house flies which were taken out from the stock cage were grouped into the females and the males by the method already described above and introduced into cylinder through the round hole 2.5 cm in diameter of the upper plate. During the experiment, this round hole is plugged up with a cork (E). Then, through the round hole 5.0 cm in diameter of the lower plate, 0.1 g of *p.p'*-DDT powder was dusted by blowing up into the apparatus with the pressure of approximately 1.5 kg/cm^2. After dusting with *p,p'*-DDT powder, the round hole of lower plate is plugged up with a rubber plug (D), and the number of knockdown individuals were counted accurately at the prescribed times. And the degree of susceptibility of test insects was evaluated by determining the speed of this knokdown caused by the toxic effect of insecticide.

A brimmed bell-shaped funnel (J) of 3.5 cm calibre shown in the left of Fig. 5 is the receptacle for the insecticidal powder. The brim is 1 cm in width. This funnel is covered with 16 mesh wire gauze (G) which is fixed upon a wooden frame (H). After 0.1 g of *p,p'*-DDT powder is placed in the funnel, it is closely fitted to the round hole of lower plate. A small glass ball (I) is placed in the bottom of the funnel to prevent the dust falling into the leg tube of the funnel. The leg of funnel is connected to the compressor and a transformer is inserted between them.

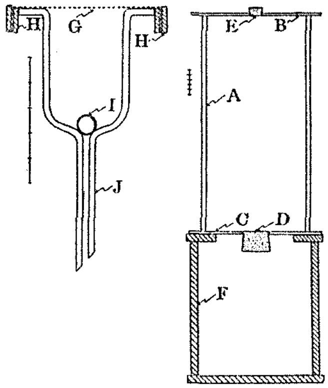

Fig. 5. Settling dust apparatus (right) and bell shaped funnel for dusting insecticidal powder (left). A, glass cylinder ; B, upper glass plate ; C, sole glass plate ; D, rubber plug ; E, cork plug ; F, apparatus holder ; G, 16 mesh wire gauze ; H, gauze holder ; I, glass boll ; J, bell shaped funnel. 1 unit of scale =1.0 cm.

RESULT AND DISCUSSION

The result of the experiment presented as the relation between time T(minutes) and the cumulated knockdown percentage Y_K for each concentration C (%) is shown in Table 3. This experiment was carried out in the period from June 10th to 30th, 1950 under the room temperature of about 30°C. The flies, knocked down as a result of intoxication by DDT, did not recover; all of them died out after several hours.

Table 3. Time T (min.)-per cent knockdown Y_K of adults of the common house fly, *Musca domestca vicina* Macq., for *p,p'*-DDT powder in the range of concentration C from 0.5 to 4.0%.

Sex		Female				Male			
Concentration		0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
Number of experiments		25	25	25	25	17	17	17	17
Number of individuals		337	312	330	305	206	246	228	214
Time	4	1.19	2.56	4.24	4.26	5.82	8.12	12.28	18.22
	6	8.31	10.58	19.09	22.30	24.76	38.21	50.88	56.08
	8	19.59	28.21	35.76	43.61	55.34	63.82	75.88	80.84
	12	49.26	60.26	63.94	73.77	85.92	91.46	96.93	98.13
	16	69.44	77.24	83.33	91.80	97.57	98.78	99.56	100.00
	24	89.91	92.31	96.06	98.36	100.00	100.00	100.00	—
	32	97.03	97.12	100.00	100.00	—	—	—	—

The writer has stated time and again that the susceptibility of the common house fly represented by the cumulated knockdown percentage as the result of toxic action of DDT, BHC and others is distributed in a nearly symmetrical sigmoid curve against the logarithms of time after treatment. This relation is also considered to hold true naturally for the data of the present experiment, By transforming the cumulated knockdown percentage Y_K to probit y_k and the time T to logarithm t, the parameters of the equation of time-knockdown regression isodoses

$$y_k = 5 + b_c(t - \bar{t}_c)$$

were calculated[2]. The results are shown in Table 4 and Fig. 6. As seen in Table 4, the regression coefficients are nearly eqaul without regard to the concentrations of the dust, both in the female and the male, respectively. The mean values of regression coefficients are 4.550 for the female and 5.961 for the male. The regression coeffici-

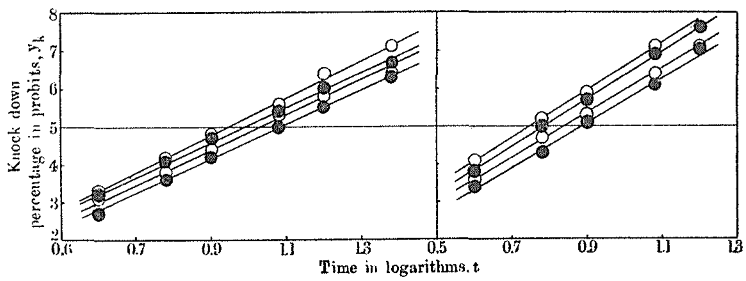

Fig. 6. Time-knockdown regression isodoses of adults of the common house fly, *Musca domestica vicina* Macq., for *p,p'*-DDT powder in the range of concentration 4.0, 2.0, 1.0 and 0.5% (top to bottom). Left, female ; right, male.

ents of the female are slightly smaller than that of the male in all the concentrations of DDT. Murray[17], Miller and Simanton[16] and Eagleson[7] reported that similar differences in regression coefficients of concentration-mortality isochrones were observed between two sexes in the experiments with pyrethrum kerosene spray carried out by the Peet-Grady method and a modified Peet-Grady method. A similar difference is also observable in the result of pyrethrum kerosene spray experiment of Eagleson[7]

Table 4. Characteristics of time-knockdown regression isodoses of adults of the common house fly, *Musca domestica vicina* Macq., for *p,p'*-DDT powder in the range of concentration C from 0.5 to 4.0%.

Sex	Concentration C	Regression coefficient b_c	Standard deviation σ_c	Log median knock down time t_c	Median knock down time $\overline{T}_C$(min.)
Female	0.5	4.481	0.223	1.08918	12.28
	1.0	4.370	0.229	1.03943	10.95
	2.0	4.382	0.228	0.98714	9.71
	4.0	4.979	0.201	0.94112	8.73
Male	0.5	5.726	0.175	0.88475	7.67
	1.0	5.817	0.172	0.83724	6.88
	2.0	6.234	0.160	0.78264	6.06
	4.0	6.067	0.165	0.75330	5.67

carried out using the spray tunnel apparatus. While the result of Eagleson's experiment agrees with the writer's result in that the regression coefficient of the female is smaller than that of the male, the relation was quite the reverse in the experimental results of Murray[17], Miller and Simanton[16]. By the topical application method, Kerr[12] obtained the result that the lines for the regression of mortality in probits on log dosage of DDT for the males and the females of the pomace fly, *Drosphila melanogaster* Meigen, were parallel.

Next, the writer wishes to consider the relation of time to concentration in the female and the male. Already, the writer frequently stated that the Ostwald's form-

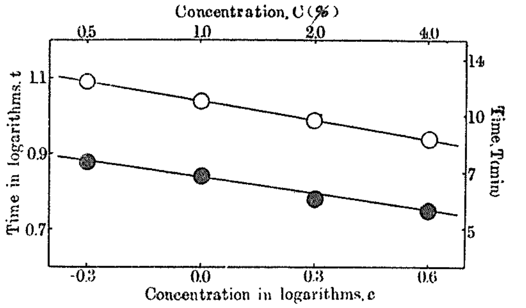

Fig. 7. Relation between log time t and log concentration c at the 50 per cent knockdown of adults of the common house fly, *Musca domestica vicina* Macq., for *p, p'*-DDT powder in the range of concentration C from 0.5 to 4.0%. The equations of the two lines in original units are $C^{0.167}t = 10.940$ for female (hollow circles and solid line) and $C^{0.149}t = 6.870$ for male (solid circles and solid line) respectively.

ula, $C^n t=k$, is applicable to the relation of these two variables in the experiments with dust of DDT or BHC using the common house fly. In this formula C is the concentration of chemical used, t is the length of exposure time in logarithms and n and k are two constants, of which the former expresses the slope and the latter the position respectively.* The results of calculation are shown in Table 5. The relation of log-exposure time to log-concentration as plotted in Fig. 7 is clearly linear so that the equation $C^n t=k$ can be applied to express this relation. Table 6 is the result of

Table 5. Relation between log time t and log concentration c at the 50 per cent knockdown of female and male adults of the common house fly, *Musca domestica vicina* Macq., for *p,p'*-DDT powder in the range of concentration C from 0.5 to 4.0%

Sex	Regression equation $t + b_2 c = a_2$ or in original units $C^n\ t = k$	Precision of parameters a_2 and b_2		
		S^2	$V(a_2)$	$V(b_2)$
Female	$t + 0.1649\ c = 1.0390261$	0.0000037	0.0000009	0.0000081
	or $C^{0.165}\ t = 10.940$		at $\bar{c} = 0.150515$	
Male	$t + 0.1491\ c = 0.8369300$	0.0000068	0.0000017	0·0000015
	or $C^{0.149}\ t = 6.870$		at $\bar{c} = 0.150515$	

Table 6. Analysis of variance for testing linearity of relation between log time t and log concentration c for the data in Table 4.

Sex	Variance due to	Degrees of freedom	Sum of squares	Mean square
Female	Rectilinear relation between t and c, the linear term	1	0.0123242	0.0123242
	Single curvature from straight line the quadratic term	1	0.0000033	0.0000033
	Error	1	0.0000040	0.0000040
	Total	3	0.0123315	--
Male	Rectilinear relation between t and c, the linear term	1	0.0100778	0.0100778
	Single curvature from straight line, the quadratic term	1	0.0000826	0.0000826
	Error	1	0.0000523	0.0000523
	Total	3	0.0102127	—

* It should not be confused with a subsequent use of the same symbol n for degrees of freedom in statistical discussion and k for knockdown which is used in this paper.

analysis of variance for testing the linearity of relation of these two variables, i. e. to examine whether we must conceive a more enlarged formula with another qudratic term or not. As is evident from the figures of Table 6, the ratio of variance for the quadratic coefficients to that for the error is less than 1.7 both in the female and the male, it is concluded that there is no necessity to apply the more enlarged formula to the relation of these two variables. As the values of n in the equations of the time-concentration regression line at the 50 per cent knockdown, $C^n t = k$, are almost equal for the female and the male, a combined regression coefficient has been computed from the sum of the numerators and the denominators for the two curves to obtain an improved value applying to both relations of the female and the male. The combined regression coefficient is $(0.1649+0.1491)/2=0.1570$ and the revised equations for the female and the male are $t+0.1570c=1.0378530$ or $C^{0.157}\ t=10.909$ and $t+0.1570c=0.838180$ or $C^{0.157}\ t=6.888$, respectively. The difference of susceptibility between the female and the male which is expressed with the ratio of the median knockdown concentration for a certain exposure time is

$$(1.0378530-0.8381180)/0.1570=1.271974$$

Thus, the male is 18.70 times as susceptible as the female. Inversely, the difference of susceptibility when expressed with the ratio of the median knockdown time at a certain concentration is $10.999/6.888=1.584$; namely, the male is 1.58 times as susceptible as the female.

The above mentioned result that the male of the common house fly is more susceptible to the knockdown effect of DDT than the female agrees with the results obtained by the previous investigators in experiments carried out using DDT and certain other toxicants.

For example, Bruce and Decker,[4] and Busvine[5] found that the females are more resistant to the toxic effect of DDT than the males in both susceptible and resistant strains. Murray,[17] Miller and Simanton,[16] Eagleson[7] using pyrethrum kerosene solution, Nagasawa and Uruha[19] using mosquitocidal incence made of pyrethrum mixed with benzophenone, Nagasawa[22] using pyrethrins, methoxychlor, parathion, toxaphene, chlordane, *p*, *p*′-DDT, γ-BHC and 1,1′ -bis (*p*-chlorophenyl) 2-nitro propane powders, and also Fay et al.[8] with the residnal deposits of pyrethrum, BHC, DDT, methoxychlor, "Q-137", DFDT, aldrin, dieldrin, heptachlor, TDE and toxaphene attained to a similar conclusion. Eagleson,[7] however, by comparing his experimental result with those of Murray,[17] Miller and Simantot,[16] found that the difference of susceptibility between the female and the male is not always the same even in the same toxicant. Also Nagasawa[22] reported that there are considerable differences in the grade of susceptibilities of the female and the male according to the kind of toxicants. This tendency was also observed by Cochran[6] in his experiments with various toxicants using the American cockroach, *Periplaneta americana* (L.).

Similar results are also observed in many other test insects. For example, Woodbury[33] observed that the males of the German cockroach, *Blatella germanica* (L.), are more susceptible to pyrethrins kerosene solution than are the females. Grayson[10] obtained a similar result in experiments with DDT and BHC in both resistant and susceptible strains of the German cockroach and he observed that the females of resistant individuals acquired an extremely great resistance whereas the males were only moderately resistant. Woodbury and Barnhardt[34] reported a similar result in the experiment with kerosene using the bed bug, *Cimex lecturarius* L. Pielou and Glasser[27] found that the females of *Macrocentrus ancylivorus* Rohw. developed greater resistance than the males. Also Munson and Gottlieb[18] obtained similar results with DDT in the American cockroach and Cockran[6] with lindane, chlordane, toxaphene, dieldrin, methoxychlor and DDT in same insect. In the investigation dealing with the relation of sexes and developmental stages to susceptibility, Ricci[28] found that the male and female nymphs and the adult females of the Oriental cockroach, *Blatta orientalis* L.,were all more resistant to DDT than the adult males. Kerr[12] also obtained a similar result with DDT in the pomace fly.

However, Utida and Harukawa[32] reported the converse result that the female azuki bean weevil, *Callosobruchus chinensis* L., was more susceptible to the lethal effect of paradichlorbenzol than the male. In the experimental result obtained by Sun[30] using the Mexican bean weevil, *Zabrotes subfasciatus* (Boh.), and the confused flour beetle, *Tribolium confusum* Duv., there was no sxual difference in susceptibility to the toxic effect of carbon disulphate. Nagasawa's result[22] obtained in experiments carried out to test the susceptibility of the common house fly to knockdown effect of parathion and methoxychlor was quite similar to the result reported by Sun[30].

Factors which may cause the sexual difference in susceptibility have been discussed by many authors. Utida and Harukawa[32] reported that tha sexual difference in susceptibility of the azuki bean weevil to naphthalen may simply be attributable to the difference in body weight. And also Kerr[12] tried to introduce the body weight for the explanation of his experimental result. Nagasawa[24] reported that, of the house flies that emerged from the same culture medium, the smaller individulas of the common house fly were more susceptible to the knockdown effect of *p,p'*-DDT powder than the larger individuals. Bliss[3] stated that the smaller silk worm larvae, *Bombyx mori* L., were killed by a smaller quantity of arsenic per body weight than the larger larvae. Therefore, the difference in body weight between the female and the male may be considered as one of the factors that affect the susceptibility to toxicants. Since the rate of respiration and metabolism is proportional to the relative body weight in almost all cases, the body weight may be of some use in the discussion of experimental results of fumigants.

However, as the quatients obtained by dividing the median lethal times of the female and the male in Table 4 with the body weights of the female and the male in Table 7, respectively, are not equal, it is clear that the body weight is not the sole factor that brings forth the difference in susceptibility between sexes. As is apparent from the data in Table 7, the difference in amount of crude fat can not be considered as the sole factor that controls the susceptibility. Munson and Gottlieb[18] found that the resistance of the American cockroach to DDT shows a high correlation with their lipid content, but there seems to be a difference in resistance between the two sexes, which is not accounted for by a difference in lipid content. According to Table 7 the content of ash is somewhat higher in the female than in the male. Though the

Table 7. Chemical analysis of the female and the male adults of the common house fly, *Musca domestica vicina* Macq. (Averege of 300 individuals).

Sex	Weight (mg.)	Moisture		Anhydrous matter		Crude fat			Ash		
		mg[1]	%[2]	mg[1]	%[2]	mg[1]	%[2]	%[3]	mg[1]	%[2]	%[3]
Female	19.75	14.62	73.43	5.29	26.57	0.57	2.88	10.83	0.30	1.53	5.76
Male	16.75	12.25	73.13	4.50	26.85	0.51	3.06	11.38	0.20	1.23	4.58

[1] Weight per living insect. [2] Per cent per living insect. [3] Per cent in anhydrous matter of an insect.

further detailed analytical study of the ash contents with reference to the relative contents of various other chemical elements seems to be necessary, the difference in ash content may be one of the factors that affects the susceptibility, since Kikkawa and Fujito,[13] Hiroyoshi[11] reported that the resistant strains of the pomace fly to toxic action of DDT generally contained a larger quantity of iron than the susceptible strains. Matsubara[15] tried to explain the difference in susceptibility to pyrethrins of two sexes of the common house fly from the standpoint of lipase activity and detoxification of pyrethrins, but the result was negative. Various other opinions which have been presented to explain the difference of susceptibility in two sexes, but almost all of them are nothing but a hypothesis. In regard to this matter, we must reserve our opinion until further investigations will have been made.

For the present, the writer desires only to consider some questions relating to the biological assay or inspection of insecticides.

On the basis of results reported above, it is evident that the numbers of the female and the male insects should be as nearly equal as possible when we carry out an experiment using population, whose sexes are not identified beforehand, to evaluate the effectiveness of a toxicant. The importance of the fact that the sex ratio in the population should be 50 % when the experiment is carried out using population whose sexes are not identified beforehand, has already been pointed out by previous authors such as Miller and Simanton,[16] Nagasawa[23,24] Simanton and Miller[29] in the case of the common house fly, Lord[14] in the pomace fly and Sun[30] in the Mexican bean weevil and the confused flour beetle.

Taking into consideration the fact that the susceptibility of house flies varies with the time elapsed after emergence, we often group the separate flies according to the date of emergence and rear them separately. But this measure may bring forth an undesirable result that the earlier emerged groups contain the males more than the females, while the later emerged ones contain the females more than the males. This situation comes about because the females have somewhat longer duration of development than the males as is evident in Table 8. Thomssen and Doner[31] pointed out

Table 8. Sexual difference in duration of development from egg to adult of the common house fly, *Musca domestica vicina* Macq. Residual products of "tofu" making were used for the culture medium of larval stage.

Day	Female		Male		$\frac{♂}{♀ + ♂} \times 100$
	Number	%	Number	%	
7	68	13.23	85	18.97	55.56
8	113	21.98	118	26.34	51.08
9	209	40.66	193	43.08	48.01
10	110	21.40	44	9.82	28.57
11	11	2.14	7	1.56	38.89
12	3	0.58	1	0.22	25.00

that the sex ratioes of the common house fly emerged in the former half of emergence period are different from those of the flies emerged in the latter half. As shown in Table 9 which is the summary of the figures of Table 8, the mean duration of development from egg to adult is somewhat longer in the female than in the male. The result of F-test shows that the difference between the mean durations of two sexes is statistically significant. $F_0 = 20.89 > F^1_{960}(0.05)$.

Table 9. Duration of development from egg to adult of the common house fly, *Musca domestica vicina* Macq. Summary of the data in Table 8.

Sex	Number of individuals	Mean (day)	Standard deviation (day)	Coefficient of variation (%)
Female	514	8.790 ± 0.045	± 1.029	11.70
Male	448	8.493 ± 0.051	± 1.085	12.78

In the test such as the Peet-Grady method in which a large number of individuals are used at a time, it is absolutely necessary that the sex ratio of the female to the male in a population emerged from a culture medium or that of population of a stock cage is equal to 1 : 1. Also, the same condition must not be ignored in the test in which a small number of individuals are used at a time and all the flies in a stock are used up for an experiment.

Table 10. Sex ratio of the common house fly, *Musca domestica vicina* Macq., from laboratory cultures. Residual products of "tofu" making were used for the culture medium of larval stage.

No. of pot	Female	Male	$\frac{♂}{♀+♂}\times 100$	$x^2(1:1)$
1	119	118	49.79	0.004
2	269	234	46.52	2.436
3	118	123	51.04	0.104
4	65	54	45.38	1.017
5	278	304	52.23	1.162
6	97	129	56.58	4.531
7	99	86	46.49	0.904
8	179	173	49.15	0.103
9	168	156	48.15	0.444
10	171	135	44.12	4.235
11	29	38	56.72	2.062
12	44	35	44.30	1.025
13	26	28	51.85	0.074
14	25	27	51.92	0.077
15	34	21	38.18	3.073
16	51	52	50.49	0.010
17	79	63	44.37	1.803
18	55	55	50.00	0.000
19	197	202	50.63	0.063
20	55	57	50.89	0.036
21	131	118	47.39	0.679
22	39	47	54.65	0.745
23	129	158	55.05	2.930
24	171	191	52.76	1.105
25	210	172	45.03	3.780

Miller and Simanton[16] reported the mean sex ratio of the populations emerged from eleven culture jars was 51.4%. The figures in Table 10 is the writer's results observed on the flies emerged from 25 culture jars. In this experiment, the residual products of "tofu" making were used for the culture medium of larval stage. Another result of observationson 10 stock cages is given in Table 11. As shown in figures of x^2-test, there are some cases in which the sex ratio is not 50%. $x^2>3.841$, $P_r=0.05$.

As has been already pointed out by Miller and Simanton[16], a considerable varia-

Table 11. Sex ratio of the common house fly, *Musca domestica vicina* Macq., in a fly cage.

No. of cage	Female	Male	$\frac{♂}{♀+♂}\times 100$	$x^2(1:1)$
1	439	506	53.54	4.750
2	335	339	49.70	0.024
3	237	271	53.35	2.276
4	283	272	49.00	0.218
5	260	291	58.08	1.744
6	306	254	45.36	4.829
7	114	187	62.13	17.449
8	237	372	73.08	29.926
9	789	719	47.68	4.035
10	111	105	48.61	0.167

tion exists between the ratioes of successive groups of flies emerged from a stock cage; therefore, we must always be careful so as to introduce the same numbers of the males and the females in the test chamber.

SUMMARY

By the settling dust apparatus method, the writer investigated the difference in susceptibilities of the sexes of the common house fly, *Musca domestica vicina* Macquardt, to the knockdown effect of *p,p′*-DDT powder. On the basis of the experiment, the writer pointed out some important factors which must be taken into consideration in the course of biological assay of insecticides. In addition, some sexual characters in the external morphology of adult stage were described.

(1) We can determine the sexes of living individuals of the adult common house fly easily and unmistakably by the ratio of frons to width of head, the pattern of abdominal tergites, the color and shapes of abdominal sternites.

(2) A marked difference in susceptibility to the effect of insecticide was found to exist between the males and the females; usually the males were more susceptible than the females. When the difference of suceptiblities between the female and the male is expressed as the ratio of the median knock down concentration for a certain exposure time, the male is 18.70 times as susceptible as the female. If the difference of susceptibility is expressed as the ratio of the median knockdown time at a certain concentration the male is 1.58 times as susceptible as the female.

(3) When the experiments for biological assay of insecticides are carried out using test insects in which the males and the females are intermingled, it is essential that the proportions of the males and the females be always equal in every tests throughout the experiment.

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