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$CuSO_4$ Resistance in *Drosophila melanogaster*

V. Maintenance of the Raised Resistibility of Copper

By

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Introduction

The present author has already reported in the first and the second papers of this series that, when a strain of *Drosophila melanogaster* Oregon RS was cultured on PEARL's medium containing sublethal dose (0.5 mM) of $CuSO_4$, the flies of the next generation showed obvious resistance to copper. The copper resistant substrain was denoted as the Cu-strain by the author (YANAGISHIMA and SUZUKI, 1959 a, b). The Cu-strain showed cross-resistance or collateral sensitivity to other bivalent metallic salts (YANAGISHIMA, 1961a). Furthermore, the Cu-strain differed not only in some ecological characters but also in some physiological activities from the normal strain (YANAGISHIMA, 1961b).

As for the acquisition of resistance of insects to a given toxic environment, many reports have been published so far, most of them insisting that the origin of resistance is attributable to the genetic changes of populations caused by random mutations and selections. On the other hand, only a few reports have been known attributing the changes of characters in some environmental changes to adaptive processes. It can be said, in short, that two theories are proposed to explain the causes of changes of population characters induced by some environmental changes.

Most investigators, having the former category of thought mentioned above, believe that resistant characters to insecticides are transmitted according to MENDEL's law, though admitting, at the same time, that in some cases the cytoplasmic inheritance may take place. But even when cytoplasmic inheritance is taken into consideration, cytoplasmic factors are considered to be able to act only under the control of nuclear genes. In some cases resistant characters are thought to be controlled by polygenes (BRUCE and DECKER, 1950; OPPENOORTH and DRESDEN, 1953; and OSHIMA, 1954), and in other cases a single gene control is supported (HARRISON, 1950, 1954; MELTZER and KIRK, 1953; TSUKAMOTO and OGAKI, 1953, 1954; LICHTWARDT, 1956), though it is not definite whether a gene controlling a resistance is recessive or dominant. Most of the investigators of

this school used the toxic environments, under which mortalities of test animals were nearly 50%. Recently, WADDINGTON (1953, 1959) proposed a new idea of "genetic assimilation", to explain the acquisition of new characters with changes of environments. This idea is, however, somewhat similar to SCHMALHAUSEN's (1949), and does not necessarily exclude selection.

The author believes that, at the present state of our knowledge, it will not be proper to settle the matter impatiently, but that critical experimental results should be accumulated before the matter is settled. From this viewpoint, the author attempted to inquire the hereditary nature of the maintenance of the copper resistance by culturing *Drosophila* successively in various media and by crossing of flies having different careers. This paper is no less than the results of these experiments.

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Experiments

I. Emergence Tests Performed during Successive Generations

Changes in some characters such as ecological behaviors and physiological activities associated with the acquisition of the copper resistance during the culture on the copper medium for one generation have already been reported by the author. The following tests were designed to know whether or not the degrees of copper resistance thus acquired might increase by further successive culture on the copper medium and also on the normal medium.

Material and Method

The strain of *Drosophila melanogaster* used in this experiment was the same as that used previously by the author and had since been stocked on PEARL's medium.

The culture medium used was PEARL's synthetic medium, on which a few drops of yeast suspension were dropped just before the experiment was started. Rearing temperature was 25°±0.1°C throughout this experiment.

The experimental procedures are as follows. Twenty 1st instar larvae were transferred into a culture bottle containing 15 ml of the culture medium on which yeast drops had been added. The larvae grown in the culture bottle pupated on the side wall. When pupae began to have eyes and wings, each pupa was transferred in order to get virgin flies, one to one, to a little tube

with a cotton plug on its upper side containing a little amount of the normal medium. When emerged, a couple of male and female flies was transferred into a oviposition tube to which a slide glass with the culture medium on it had been inserted. Eggs were laid on the slide glass.

The larvae hatched from these eggs were divided into 6 groups, of which three groups were transferred to the bottles, each containing PEARL's normal medium and the other three groups were transferred to the bottles, each having PEARL's medium containing 0.5 mM $CuSO_4$. Thus three normal strains and three copper (Cu) strains were started. In order to maintain the strains, twenty 1st instar larvae were always transplanted in each bottle, and more than five bottles were used to maintain each strain. When the emergence rate had to be tested, about 10 culture bottles were used at the same time and 20 1st instar larvae were transplanted in each one of these bottles.

Figure 1 shows the pedigrees and the test plans of one of the normal and copper strains respectively as representatives of the whole schedule. At the start of this experiment, 10 culture bottles of the normal medium were prepared and the emergence rate was measured. This test is denoted as 0-1. Then a part of the larvae were transferred to the medium containing 0.5 mM of $CuSO_4$, and the emergence rate was measured (the result is denoted as 1-1). Then these adults were made to deposit eggs on the normal medium, and the 1st instar larvae hatched from these eggs were divided into three groups, of which one was transferred to 10 bottles, each having PEARL's medium containing 4 mM $CuSO_4$ and the emergence rate was measured (the result is denoted as 2-2). The second group was transferred to 10 bottles, each having 0.5 mM $CuSO_4$ containing PEARL's medium. The number of adults (designated as 0.5^2) emerged from these bottles was counted (the result is denoted as 2-1), and after counting, they were put into a oviposition tube, and under this condition further procedures of maintaining the strain and testing the emergence rate were continued. The third group was transplanted in 10 bottles of normal PEARL's medium and the number of adults (designated as 0^1) emerged from these bottles was counted (the results is shown as 2-3). Further procedures were performed following the second group mentioned above.

Other procedures shown in Fig. 1 follow the above description. All test plans are somewhat complicated as are shown in this figure; but in order to get assurance about the results of the experiments, the author dared to take these steps.

The method used in the emergence test was the same one as used in the previous work (YANAGISHIMA, 1961a). That the emergence rate in PEARL's medium containing 0.5 mM $CuSO_4$ is not different from that in the normal medium has repeatedly been ascertained; namely 0.5 mM $CuSO_4$ is surely the sublethal dose for *Drosophila melanogaster* used in this experiment. The emergence rate in PEARL's medium containing 4 mM $CuSO_4$ is about 50%; accordingly this dose was adopted as indicating ED_{50} dose.

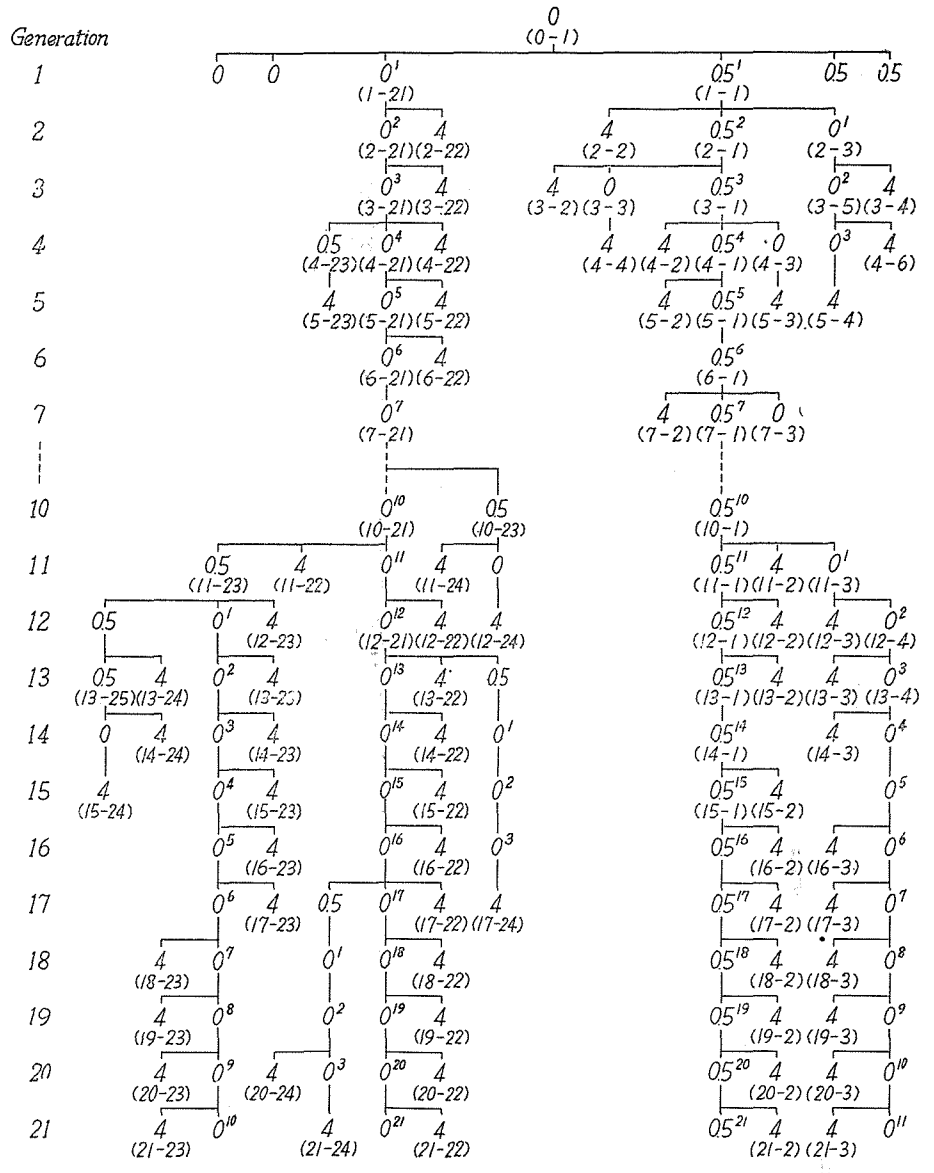

Fig. 1. Scheme illustrating an example of pedigree and test plan of one of the normal strains and the copper strains respectively. The normal strains were maintained on PEARL's medium and the copper strains were cultured successively by PEARL's medium containing 0.5 mM $CuSO_4$. 0: normal PEARL's medium; 0.5: PEARL's medium containing 0.5 mM $CuSO_4$; 4: that of containing 4 mM $CuSO_4$ (ED_{50} medium). Initial figures in parentheses show numbers of generations, which are followed by figures indicating numbers of experiments. Figures suffixed at heads of 0 and 0.5 are numbers of generations passed on respective media. Other explanations see text.

Results

The results are shown in Tables 1 and 2. As is shown in these tables, there is no statistically significant difference in the emergence rates between Cu-strain and the control strain. This is clear when we compare the emergence rate of a certain generation in 0.5 mM $CuSO_4$ containing medium with that of the corresponding generation in the normal medium. Consequently, we cannot insist that any effects of selection had surely partaken in the course, even when any changes were induced.

Now, let us compare the result of (2-2) with that of (2-22). It is clear that the emergence rate of the former is much higher than the latter, which shows that the resistance to copper of ED_{50} dose is gained through the life of only one generation in sublethal copper containing medium. Statistic examination showed that this difference was highly significant ($0.001 > \alpha$).

Then three questions may arise; first, "Does this phenomenon appear always in the same manner when we set up the same kind of experiment?" Secondly, "How many generations does this change in resistance persist, i.e., how is the stability of the change of resistant character?" Thirdly, "Does this rise in resistance to copper increase or decrease with the following successive culture in the sublethal copper medium?"

We can find answers to these questions when we carefully examine Tables 1 and 2. As for the first question it may be answered as follows. The acquisition of resistance is always realized when the larvae are reared in the sublethal copper medium for only one generation. This is clear when we compare the results of (5-23) and (11-24) with the corresponding results of (5-22) and (11-22), or the result of (12-23) with that of (12-22).

We can find the answer to the second question as to the stability of the changed character by comparing the series of results (12-22, 13-22, 14-22, 15-22, 16-22, 17-22, 18-22, 19-22, 20-22, 21-22) with the series of corresponding results (12-23, 13-23, 14-23, 15-23, 16-23, 17-23, 18-23, 19-23, 20-23, 21-23). The former series is the control series, which had never met with copper, except when the individuals to be used in the experiments were subjected to 4 mM $CuSO_4$ containing test medium. The latter is the series of experiments, obtained by using the descendants whose ancestors (the 11th generation calculated from the beginning of the experiment) had only once passed in 0.5 mM $CuSO_4$ containing medium. The result is very clear, namely, with respect to all corresponding combinations of the results, the emergence rates are higher in the latter series than in the former series. At six cases out of ten, the differences are statistically significant. In other words, the flies that experienced the sublethal dose of copper for only one generation can gain the hereditary character of resistance to copper, and this character transmits more than ten generations.

The answer to the third question may be found in the following experiments. If the larvae whose ancestors had experienced the medium containing

Table 1. Emergence rates at the experiments shown in Fig. 1.
Figures in parentheses show numbers of bottles.

Exp. No. in Fig. 1	Career	Test medium	No. of larvae	Emerged No.	%
0–1	0	0	200(10)	187	93.5
1–1	0.5	0.5	200(10)	183	91.5
1–21	0	0	200(10)	180	90.0
2–1	0.5^2	0.5	200(10)	180	90.0
2–2	0.5–4	4	160(8)	128	80.0
2–3	0.5–0	0	100(5)	90	90.0
2–21	0^2	0	200(10)	179	89.5
2–22	0	4	360(18)	198	55.0
3–1	0.5^3	0.5	180(9)	163	90.6
3–2	0.5^2–4	4	200(10)	138	69.0
3–3	0.5^2–0	0	180(9)	165	91.7
3–4	0.5–0–4	4	180(9)	160	88.9
3–21	0^3	0	80(4)	72	90.0
3–22	0^2–4	4	240(12)	106	44.2
4–1	0.5^4	0.5	360(18)	324	90.0
4–2	0.5^3–4	4	180(9)	110	61.1
4–3	0.5^3–0	0	200(10)	184	92.0
4–4	0.5^2–0–4	4	120(6)	80	66.7
4–6	0.5–0^2–4	4	200(10)	121	60.5
4–21	0^4	0	200(10)	170	85.0
4–22	0^3–4	4	180(9)	87	48.3
4–23	0^3–0.5	0.5	200(10)	190	95.0
5–1	0.5^5	0.5	180(9)	160	88.9
5–2	0.5^4–4	4	260(13)	180	69.2
5–3	0.5^3–0–4	4	400(20)	299	74.8
5–4	0.5–0^3–4	4	200(10)	129	64.5
5–21	0^5	0	200(10)	196	98.0
5–22	0^4–4	4	400(20)	221	55.3
5–23	0–0.5–4	4	160(8)	120	75.0
6–1	0.5^6	0.5	180(9)	165	91.7
6–21	0^6	0	200(10)	190	95.0
6–22	0^5–4	4	80(4)	40	50.0
7–1	0.5^7	0.5	100(5)	90	90.0
7–2	0.5^6–4	4	380(19)	267	70.2
7–3	0.5^6–0	0	200(10)	184	92.0
7–21	0^7	0	200(10)	166	83.0
10–1	0.5^{10}	0.5	100(5)	90	90.0
10–21	0^{10}	0	200(10)	186	93.0
10–23	0^9–0.5^1	0.5	200(10)	183	91.5
11–1	0.5^{11}	0.5	200(10)	181	90.5
11–2	0.5^{10}–4	4	100(5)	70	70.0
11–3	0.5^{10}–0	0	200(10)	182	91.0
11–22	0^{10}–4	4	200(10)	111	55.5
11–23	0^{10}–0.5	0.5	200(10)	187	93.5
11–24	0–0.5–4	4	100(5)	66	66.0
12–2	0.5^{11}–4	4	200(10)	116	58.0
12–3	0.5^{10}–0–4	4	140(7)	91	65.0
12–4	0.5^{10}–0^2	0	200(10)	185	92.5
12–21	0^{12}	0	200(10)	196	98.0
12–22	0–4	4	100(5)	57	57.0
12–23	0.5–4	4	120(6)	78	65.0
12–24	0.5–0–4	4	200(10)	118	59.0
13–2	0.5^{12}–4	4	100(5)	72	72.0
13–4	0.5^{10}–0^3	0	100(5)	90	90.0
13–3	0.5^{10}–0^2–4	4	200(10)	154	77.0
13–22	0–4	4	200(10)	76	38.0
13–23	0.5–0–4	4	240(12)	154	64.2
13–24	0–0.5^2–4	4	200(10)	128	64.0
14–3	0.5^{10}–0^3–4	4	140(7)	105	75.0
14–22	0	0	200(10)	108	54.0
14–23	0.5–0^2–4	4	200(10)	121	60.5
14–24	0.5^2–0–4	4	120(6)	80	66.7
15–2	0.5^{14}–4	4	200(10)	143	71.5
15–22	0–4	4	100(5)	56	56.0
15–23	0.5–0^3–4	4	320(16)	197	61.6
15–24	0.5^3–0–4	4	400(20)	299	74.8
16–2	0.5^{15}–4	4	200(10)	143	71.5
16–3	0.5^{10}–0^5–4	4	240(12)	172	71.7
16–22	0–4	4	140(7)	69	49.3
16–23	0.5–0^4–4	4	240(12)	150	62.5
17–2	0.5^{16}–4	4	160(8)	100	62.5
17–3	0.5^{10}–0^6–4	4	240(12)	145	60.4
17–22	0–4	4	120(6)	67	55.8
17–23	0.5–0^5–4	4	240(12)	165	68.8
17–24	0.5–0^3–4	4	320(16)	199	62.2
18–3	0.5^{10}–0^7–4	4	200(10)	141	70.5
18–2	0.5^{17}–4	4	160(8)	108	67.5
18–22	0–4	4	140(7)	69	49.3
18–23	0.5–0^6–4	4	200(10)	136	68.0
19–2	0.5^{18}–4	4	220(11)	145	65.9
19–3	0.5^{10}–0^8–4	4	240(12)	149	62.1
19–22	0–4	4	200(10)	110	55.0
19–23	0.5–0^7–4	4	200(10)	131	65.5
20–2	0.5^{19}–4	4	260(13)	169	65.0
20–3	0.5^{10}–0^9–4	4	200(10)	138	69.0
20–22	0–4	4	200(10)	109	54.5
20–23	0.5–0^8–4	4	500(25)	302	60.4
21–2	0.5^{20}–4	4	380(19)	233	61.4
21–3	0.5^{10}–0^{10}–4	4	200(10)	133	66.5
21–22	0–4	4	200(10)	102	51.0
21–23	0.5–0^9–4	4	200(10)	122	61.0
21–24	0.5–0^3–4	4	200(10)	124	62.0

Table 2. Examinations of differences among the results shown in Table 1. Examinations of differences between various combinations of results (χ^2-test).

(0- 1) : (1-21)	$0.95 > \alpha > 0.90$
(1- 1) : (2- 1)	$0.80 > \alpha > 0.70$
(1- 1) : (1-21)	$0.80 > \alpha > 0.70$
(0- 1) : (1- 1)	$0.70 > \alpha > 0.50$
(2- 1) : (2-21)	$\alpha > 0.99$
(2- 1) : (1-21)	equal
(2- 3) : (2-21)	$0.95 > \alpha > 0.90$
(2- 2) : (2-22)	$0.001 > \alpha$ *
(1-21) : (2-21)	$\alpha > 0.99$
(2- 1) : (3- 1)	$\alpha > 0.99$
(3- 3) : (3-22)	$0.001 > \alpha$ *
(3- 1) : (3-21)	$0.95 > \alpha > 0.90$
(3- 2) : (3-22)	$0.001 > \alpha$ *
(3- 1) : (3-21)	$0.95 > \alpha > 0.90$
(4- 2) : (4-22)	$0.02 > \alpha > 0.01$*
(4- 3) : (3- 4)	$0.90 > \alpha > 0.80$
(4- 1) : (3- 1)	$0.98 > \alpha > 0.95$
(4- 6) : (3- 4)	$0.001 > \alpha$ *
(4- 6) : (4- 2)	$0.99 > \alpha > 0.98$
(4-21) : (4-23)	$0.01 > \alpha > 0.001$*
(4- 6) : (4-22)	$0.05 > \alpha > 0.02$*
(3-21) : (4-23)	$0.001 > \alpha$ *
(4- 1) : (4-23)	$0.10 > \alpha > 0.05$
(5- 4) : (3- 3)	$0.001 > \alpha$ *
(5- 4) : (4- 6)	$0.50 > \alpha > 0.30$
(5- 4) : (2- 2)	$0.01 > \alpha > 0.001$*
(5- 4) : (5- 3)	$0.10 > \alpha > 0.05$
(5- 4) : (5-22)	$0.05 > \alpha > 0.02$*
(5- 1) : (5-21)	$0.001 > \alpha$ *
(5-22) : (5-23)	$0.001 > \alpha$ *
(6- 1) : (6-21)	$0.30 > \alpha > 0.20$
(7- 1) : (7-21)	$0.20 > \alpha > 0.10$
(10-21) : (10- 1)	$0.50 > \alpha > 0.30$
(10- 1) : (10-23)	$0.95 > \alpha > 0.90$
(10-21) : (10-23)	$0.80 > \alpha > 0.70$
(11- 1) : (11- 3)	equal
(11-24) : (11-22)	$0.70 > \alpha > 0.50$
(11- 2) : (11-22)	$0.05 > \alpha > 0.02$*
(11-24) : (11- 2)	$0.20 > \alpha > 0.10$
(11-23) : (11- 1)	$0.50 > \alpha > 0.30$
(12- 3) : (12- 2)	$0.30 > \alpha > 0.20$
(11- 3) : (12- 4)	$0.80 > \alpha > 0.70$
(12- 2) : (12-24)	$0.95 > \alpha > 0.90$
(12-24) : (12-22)	$0.90 > \alpha > 0.80$
(12- 3) : (12-24)	$0.50 > \alpha > 0.30$
(12-23) : (12- 2)	$0.30 > \alpha > 0.20$
(12- 3) : (12-22)	$0.30 > \alpha > 0.20$
(12- 2) : (12-22)	$0.98 > \alpha > 0.95$
(12-23) : (12-22)	$0.30 > \alpha > 0.20$
(13- 2) : (13-24)	$0.30 > \alpha > 0.20$
(13-23) : (13-22)	$0.001 > \alpha$ *
(13- 3) : (13- 2)	$0.50 > \alpha > 0.30$
(13-23) : (13- 3)	$0.01 > \alpha > 0.001$*
(13- 2) : (13-22)	$0.001 > \alpha$ *
(13-22) : (13- 3)	$0.001 > \alpha$ *
(14-24) : (14-22)	$0.05 > \alpha > 0.02$*
(14-22) : (14-23)	$0.30 > \alpha > 0.20$
(5- 4) : (14- 3)	$0.05 > \alpha > 0.02$*
(14-24) : (4- 6)	$0.50 > \alpha > 0.30$
(13- 3) : (14-23)	$0.001 > \alpha$ *
(14- 3) : (14-22)	$0.001 > \alpha$ *
(15-24) : (15-22)	$0.001 > \alpha$ *
(15- 2) : (15-24)	$0.50 > \alpha > 0.30$
(15-23) : (14- 3)	$0.01 > \alpha > 0.001$*
(15- 2) : (15-22)	$0.02 > \alpha > 0.01$*
(15-22) : (15-23)	$0.50 > \alpha > 0.30$
(16- 3) : (16-22)	$0.001 > \alpha$ *
(16-23) : (16-22)	$0.02 > \alpha > 0.01$*
(16- 3) : (16-22)	$0.001 > \alpha$ *
(17-24) : (17-23)	$0.20 > \alpha > 0.10$
(17- 2) : (17-24)	$0.98 > \alpha > 0.95$
(17-24) : (17-22)	$0.30 > \alpha > 0.20$
(17- 2) : (17-22)	$0.50 > \alpha > 0.30$
(17-23) : (17-22)	$0.05 > \alpha > 0.02$*
(17-23) : (16- 3)	$0.70 > \alpha > 0.50$
(17-22) : (17- 3)	$0.50 > \alpha > 0.30$
(18- 2) : (18-22)	$0.01 > \alpha > 0.001$*
(18- 3) : (18-22)	$0.001 > \alpha$ *
(18-23) : (18-22)	$0.001 > \alpha$ *
(19- 2) : (19-22)	$0.05 > \alpha > 0.02$*
(19-23) : (19-22)	$0.05 > \alpha > 0.02$*
(19- 2) : (19-23)	$0.99 > \alpha > 0.98$
(18- 3) : (19-23)	$0.50 > \alpha > 0.30$
(19- 3) : (19-22)	$0.20 > \alpha > 0.10$
(14-23) : (20-24)	$0.30 > \alpha > 0.20$
(20-24) : (20-22)	$0.05 > \alpha > 0.02$*
(20-24) : (20- 2)	$0.80 > \alpha > 0.70$
(20- 3) : (20-22)	$0.05 > \alpha > 0.02$*
(20-23) : (20-22)	$0.01 > \alpha > 0.001$*
(20- 3) : (21-23)	$0.50 > \alpha > 0.30$
(20- 2) : (20-22)	$0.05 > \alpha > 0.02$*
(21- 2) : (21-22)	$0.05 > \alpha > 0.02$*
(21-23) : (20- 3)	$0.50 > \alpha > 0.30$
(21-24) : (21-22)	$0.05 > \alpha > 0.02$*
(21-23) : (21-22)	$0.10 > \alpha > 0.05$
(21-22) : (21- 3)	$0.01 > \alpha > 0.001$*

Examinations of differences between various combinations of two series (F-test).

(12- 3)~(21- 3) : (12-22)~(21-22)	$0.01 > \alpha > 0.001$*
(12-23)~(21-23) : (12-22)~(21-22)	$0.001 > \alpha$ *
(12-23)~(21-23) : (12- 3)~(21- 3)	$0.001 > \alpha$ *
(12-23)~(21-23) : (12- 2)~(21- 2)	$0.20 > \alpha > 0.10$
(12-22)~(21-22) : (12- 2)~(21- 2)	$\alpha = 0.01$*
(12- 2)~(21- 2) : (12- 3)~(21- 3)	$\alpha > 0.20$

* Significant.

0.5 mM $CuSO_4$ for only one generation showed less resistance to 4 mM $CuSO_4$ (ED_{50} dose) containing medium than the larvae whose ancestors had experienced the said medium during several generations, we will be able to say that the resistant character was increased by the successive life in the copper medium containing sublethal dose of $CuSO_4$ (0.5 mM). That this is really the case can be shown when we compare the series of results (12-3, 13-3, 14-3, 16-3, 17-3, 18-3, 19-3, 20-3, 21-3, this series of results will be designated as 0.5^{10}-0 series hereafter), whose ancestors had experienced 0.5 mM $CuSO_4$ containing medium for ten generations, with another series of corresponding results (12-23, 13-23, 14-23, 16-23, 17-23, 18-23, 19-23, 20-23, 21-23; this series will be designated as 0.5^{1}-0 series hereafter), whose ancestors had experienced the said medium for only one generation (at the 11th generation in Fig. 1). This comparison can clearly be made by comparing relative dominances in resistance against the copper medium (ED_{50} medium) of the above two series to the corresponding results of the control series (12-22, 13-22, 14-22, 16-22, 17-22, 18-22, 19-22, 20-22, 21-22). Of course, when we compare the results at each corresponding generation, the emergence rates are higher at both experimental series than the control series. However, the differences may be said as greater between the control series and the 0.5^{10}-0 series than between the control series and the 0.5^{1}-0 series. That is to say, in the comparison of the former case, there are five combinations out of all nine combinations showing statistically significant difference at less than 1% level (among these, four combination are significant at less than 0.1% level), whereas, in the comparison of the latter case, there are only three combinations out of nine showing the said difference (among these, two combinations are significant at less than 0.1% level). After all, it may be said that the flies seem to increase the resistance to copper as they live longer in the sublethal copper medium. This is the answer to the third question.

Concerning this phenomenon, the author wishes to point out the following fact. The increasing of resistance with the increasing of duration of life in the sublethal copper medium can only be brought to light when the flies that had been cultured successively in the sublethal copper medium were brought to normal PEARL's medium and reared there successively. We can not demonstrate this increasing of resistance so long as the above strain (reared successively in the sublethal copper medium) is continued to remain in the same sublethal copper medium and the resistant character is tested in the 4 mM $CuSO_4$ containing medium. This is shown in the scarcely changed results of the series of experiment (2-2, 3-2, 4-2, 5-2, 7-2, 11-2, 12-2, 13-2, 15-2, 16-2, 17-2, 18-2, 19-2, 20-2, 21-2). The emergence rates of this series of experiment usually remained between 60% and 70%, though they fluctuate from one generation to another.

II. Photokinesis Experiment (The Activity Test)

It has been reported that in the male flies, there was a marked difference in the photokinesis between the normal strain and the copper resistant variant established by the training on the sublethal copper medium for one generation, but in the female flies, there was no difference in it between the two. In the present paper, some experimental results dealing with the change of behavior caused by successive copper training on the sublethal copper medium (0.5 mM $CuSO_4$-containing medium) and the durability of this change when they are returned to the normal culture medium are described.

Material and Method

The methods used in this experiment were the same as those used in the previous reports (YANAGISHIMA, 1961a). The flies used in this experiment were as follows (refer to Fig. 1).

0.5^2-flies: the flies which has been cultured on the sublethal copper medium for two generations.

0.5^{10}-0^5-flies: the flies that, after having been reared on the copper medium for ten generations, were cultured on the normal medium from the eleventh generation to the fifteenth generation.

0.5^{15}-flies: the flies cultured on the copper medium continuously for fifteen generations.

Results

The results of the activity test are shown in Table 3. It is clear from Table 3 that there are no significant differences in the activity among the female flies having various careers, though, in the case of male flies, the photokinesis activity becomes vigorous if they are once experienced in the sublethal copper medium.

In both male and female flies, there were not any significant differences between the flies cultured on the 0.5 mM copper medium for fifteen generations successively and those cultured for only one generation ($\alpha > 0.20$). 0.5^{10}-0^5 flies did not differ regardless of sex in photokinesis activities from 0.5^{15} flies ($\alpha > 0.20$).

Once the activity is changed, the numbers of generation during which the copper resistant male flies had been cultured on the copper medium had no relation to the result of the photokinesis test, and this is the parallel phenomenon to the case of acquiring copper resistance. It is of great interest that the changes in photokinesis behavior, gained associated with the acquisition of the copper resistance, are not lost even after returned to the normal medium. It is also an important fact that the above-mentioned difference was found only in male flies, and in female flies no significant change in photokinesis has been evoked by the culture in the 0.5 mM copper medium.

Table 3. Results of activity test performed with some flies shown in Fig. 1. Figures show averages of numbers of individuals which passed across the three mark lines of the test tube during one minute.

No. of Experiment	0.5^2-flies		0.5^{15}-flies		0.5^{10}–0^5 flies		control		0.5^1-flies	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	1.80	0.72	2.20	1.15	2.22	1.90	1.26	1.24	1.72	0.58
2	2.01	1.08	1.52	1.50	0.80	1.41	1.06	2.76	2.10	1.08
3	2.08	2.20	1.96	0.32	2.12	1.26	0.94	0.82	2.16	2.16
4	3.33	2.28	2.92	2.50	3.40	2.40	2.16	2.38	3.26	2.32
5	2.69	2.09	2.83	2.00	2.82	1.93	2.82	2.22	2.74	1.88
6	3.10	2.20	3.10	1.98	3.23	2.30	2.06	1.98	3.08	2.28
Average	2.50	1.76	2.42	1.57	2.43	1.86	1.71	1.89	2.50	1.71

Examinations of differences among strains (F-test).

	0.5^{15} : 0.5^{10}–0^5	0.5^2 : 0.5^{15}	control : 0.5^1	0.5^1 : 0.5^{15}
♂	$\alpha > 0.20$	$\alpha > 0.20$	$0.05 > \alpha > 0.01$*	$\alpha > 0.20$
♀	$\alpha > 0.20$	$\alpha > 0.20$	$\alpha > 0.20$	$\alpha > 0.20$

* Significant.

III. Physiological Changes during Successive Culture

Material and Method

Effects of successive culture in 0.5 mM $CuSO_4$ on physiological activities, such as resistance to other bivalent salts than $CuSO_4$ and reaction to enzyme inhibitors, were studied.

Culture media containing various metallic salts (4 mM $CuSO_4$, 15 mM $MnSO_4$, 0.5 mM $CoSO_4$, 5 mM $NiSO_4$, 20 mM $ZnSO_4$ and 0.07 mM $CdSO_4$) or various enzyme inhibitors (NaF, 2, 4-dinitrophenol, As_2O and *p*-chloromercuribenzoate) were used for the emergence test. The concentration of each salt was the median (50%) emergence dose (ED_{50}). 0.5^{20} designate the flies which had been cultured on the sublethal copper medium for twenty generations successively, and the flies cultured on the normal medium in parallel were used as the control. The 1st instar larvae from the eggs laid by 0.5^{20} flies or the control (normal) flies were transferred to various kinds of test media different in their salt or inhibitor contents, and the emergence rates were measured.

Results

a. *Resistance to bivalent metallic salts other than $CuSO_4$.*

The method used in this test was the same as that used in the previous work (YANAGISHIMA, 1961b).

The results of the test of the emergence rate are shown in Table 4, from which we can see the following facts. The emergence rates of 0.5^{20} flies on the media containing $CuSO_4$, $NiSO_4$ or $ZnSO_4$ were higher than that of the control flies ($CuSO_4$ $0.01>\alpha>0.001$, $NiSO_4$ $0.05>\alpha>0.02$, $ZnSO_4$ $0.05>\alpha>0.02$). On the contrary, 0.5^{20} flies were more sensitive to $CoSO_4$ than the control ($0.01>\alpha>0.001$). There is no difference in emergence rates from the media containing $MnSO_4$ or $CdSO_4$ between 0.5^{20} flies and the control flies ($MnSO_4$ $0.80>\alpha>0.70$, $CdSO_4$ $0.50>\alpha>0.30$).

The differences in emergence rates on the media, containing the salts

Table 4. Emergence rates in media different in the contents of the bivalent metallic salts. The careers of the material flies are shown in Fig. 1. As for the data concerning with 0.5^1-flies refer to the IIIrd report.

Strain / Test medium	$0.5^{2)}$(Cu)-flies					Control		
	No. of larvae	Pupated No.	%	Emerged No.	%	No. of larvae	Emerged No.	%
$CuSO_4$	200(10)	128	64.0	127	63.5	200(10)	112	56.0
	380(19)			255	67.1	280(14)	163	58.2
Total	580(29)			382	65.9	480(24)	275	57.3
$MnSO_4$	160(8)	138	86.3	97	60.6	200(10)	94	47.0
	200(10)			106	53.0	240(12)	148	61.7
Total	360(18)			203	56.4	440(22)	242	55.0
$CoSO_4$	200(10)			109	54.5	200(10)	142	71.0
	200(10)			97	48.5	200(10)	110	55.0
Total	400(20)			206	51.5	400(20)	252	63.0
$NiSO_4$	160(8)	99	61.9	96	60.0	200(10)	98	49.0
	200(10)			112	56.0	100(5)	51	51.0
Total	360(18)			208	57.8	300(15)	149	49.7
$ZnSO_4$	160(8)			92	57.5	200(10)	97	48.5
	200(10)			118	59.0	140(7)	72	51.4
Total	360(18)			210	58.3	340(17)	169	49.7
$CdSO_4$	140(7)	112	80.0	72	51.4	200(10)	120	60.0
	200(10)			97	48.5	200(10)	92	46.0
Total	340(17)			169	49.7	400(20)	212	53.0

Examinations of differences (χ^2-test).

Between $0.5^{2)}$(Cu)-flies and control		Between 0.5^{20} (Cu)-flies and 0.5^1(Cu)-flies	
$CuSO_4$	$0.01>\alpha>0.001$*	$CuSO_4$	$0.70>\alpha>0.50$
$MnSO_4$	$0.80>\alpha>0.70$	$MnSO_4$	$0.99>\alpha>0.98$
$CoSO_4$	$0.01>\alpha>0.001$*	$CoSO_4$	$0.50>\alpha>0.30$
$NiSO_4$	$0.05>\alpha>0.02$*	$NiSO_4$	$0.80>\alpha>0.70$
$ZnSO_4$	$0.05>\alpha>0.02$*	$ZnSO_4$	$0.20>\alpha>0.10$
$CdSO_4$	$0.50>\alpha>0.30$	$CdSO_4$	$0.95>\alpha>0.90$

* Significant.

Table 5. Results of inhibitor tests using 0.5^{20}–flies and control flies. As for the data concerning with 0.5^{1}–flies refer to the IIIrd report.

Inhibitor	Concentration m Mol	0.5^{20}–flies No. of larvae	0.5^{20}–flies Emerged No.	0.5^{20}–flies Emerged %	Control No. of larvae	Control Emerged No.	Control Emerged %
NaF	2	140	81	57.8	140	94	67.1
		120	81	67.5	140	92	65.7
	Total	260	162	62.3	280	186	66.4
	3	140	42	30.0	140	57	40.7
		140	37	26.4	140	48	34.3
	Total	280	79	28.2	280	105	37.5
	4	140	18	12.8	140	30	21.4
		140	11	7.8	140	22	15.7
	Total	280	29	10.3	280	52	18.6
2, 4-dinitrophenol	0.1	140	112	80.0	140	125	89.3
		140	109	77.9	120	109	90.8
	Total	280	211	75.4	260	234	90.6
	0.2	140	69	49.3	140	64	45.7
		140	54	38.6	140	56	40.0
	Total	280	123	43.9	280	120	42.9
	0.3	140	42	30.0	140	40	28.6
		140	29	20.7	140	33	23.6
	Total	280	71	25.4	280	73	26.1
As_2O_3	0.50	160	91	56.9	140	87	62.1
		140	78	55.7	140	98	70.0
	Total	300	169	56.3	280	185	66.0
	0.60	160	36	22.5	140	50	35.7
		140	17	12.1	140	38	27.1
	Total	300	53	17.7	280	88	31.4
	0.75	160	—	—	140	3	2.1
		140	1	0.7	140	4	2.9
	Total	300	1	0.3	280	7	2.5
P.C.M.B.	0.015	160	144	90.0	200	180	90.0
		140	118	84.3	140	131	93.6
	Total	300	262	87.3	340	311	91.5
	0.020	140	127	90.7	200	176	88.0
		140	120	85.7	140	113	80.7
	Total	280	247	88.2	340	289	85.0
	0.025	160	122	76.3	200	168	84.0
		140	108	77.1	120	96	80.0
	Total	300	230	76.7	320	264	82.5

Examinations of differences between 0.5^{20}–flies and control flies (χ^2–test).

NaF	2	$0.50 > \alpha > 0.30$
	3	$0.05 > \alpha > 0.02$*
	4	$0.01 > \alpha > 0.001$*
	a.d.**	$0.10 > \alpha > 0.05$
As_2O_3	0.50	$0.05 > \alpha > 0.02$*
	0.60	$0.001 > \alpha$*
	0.75	$0.10 > \alpha > 0.05$
	a.d.**	$0.10 > \alpha > 0.05$
2, 4-dinitrophenol	0.10	$0.001 > \alpha$*
	0.20	$0.90 > \alpha > 0.80$
	0.30	$0.95 > \alpha > 0.90$
	a.d.**	$0.50 > \alpha > 0.30$
P.C.M.B.	0.015	$0.20 > \alpha > 0.10$
	0.020	$0.30 > \alpha > 0.20$
	0.025	$0.10 > \alpha > 0.05$
	a.d.**	$0.70 > \alpha > 0.50$

Examinations of differences between 0.5^{20}–flies between 0.5^{1}–flies (χ^2–test).

NaF	2	$0.70 > \alpha > 0.50$
	3	$0.50 > \alpha > 0.30$
	4	$0.98 > \alpha > 0.95$
	a.d.**	$0.80 > \alpha > 0.70$
As_2O_3	0.50	$0.90 > \alpha > 0.80$
	0.60	$0.95 > \alpha > 0.90$
	0.75	$0.70 > \alpha > 0.50$
	a.d.**	$0.98 > \alpha > 0.95$
2, 4-dinitrophenol	0.10	$0.20 > \alpha > 0.10$
	0.20	$0.50 > \alpha > 0.30$
	0.30	$0.50 > \alpha > 0.30$
	a.d.**	$0.30 > \alpha > 0.20$
P.C.M.B.	0.015	$0.95 > \alpha > 0.90$
	0.020	$0.95 > \alpha > 0.90$
	0.025	$0.50 > \alpha > 0.30$
	a.d.**	$0.95 > \alpha > 0.90$

* Significant. ** a.d.=All data at the same time.

mentioned above, are not statistically significant between 0.5^{20} flies and 0.5^{1} flies. As 0.5^{1} flies were copper resistant flies cultured on the sublethal copper medium for only one generation, it can be said that the number of generations lived on the copper medium had no special effect on increasing or decreasing of the cross resistance or collateral sensitivity to other bivalent salts.

b. *Enzyme inhibitor test.*

Whether the reactions of the copper resistant flies to some enzyme inhibitors might change during the successive culture on the sublethal copper medium or not was tested, using NaF (2, 3 and 4 mM), 2, 4-dinitrophenol (0.1, 0.2 and 0.3 mM), As_2O_3 (0.5, 0.6 and 0.75 mM) and *p*-chloromercuribenzoate (0.015, 0.02 and 0.025 mM). The same methods as used in the previous tests (YANAGISHIMA, 1961b) were employed to observe the emergence rates. The numbers of individuals and culture bottles used are shown in Table 5.

The results are shown in Table 5. In the case of NaF, 0.5^{20} flies showed usually lower emergence rates than the control flies, regardless of the test concentrations, though statistical significances were different in each concentration. This means that 0.5^{20} flies are more sensitive to NaF than the control. In the test with 2, 4-dinitrophenol, 0.5^{20} flies showed lower emergence rate than the control flies at 0.1 mM (highly significant), but there were no differences at 0.2 and 0.3 mM. When As_2O_3 was added to the test medium, 0.5^{20} flies showed lower emergence rate than the control flies, in which the differences were statistically significant in the cases of 0.5 and 0.6 mM. There were no significant differences in the emergence rates on the media containing three kinds of concentrations of *p*-chloromercuribenzoate.

It is interesting that there were no significant differences in the above-mentioned metabolic changes between 0.5^{20} flies and 0.5^{1} flies. Here again it was confirmed that the acquired characters appeared in association with the copper resistance did not change, irrespective of how long the copper resistant substrain might be cultured on the sublethal copper medium.

After all, it can be said that the changes of metabolic activities observed in 0.5^{2} flies have been maintained unchanged during the successive culture of twenty generations (till 0.5^{20} flies) in the same sublethal copper medium.

IV. Cross experiment

In order to get thorough knowledge about the hereditary nature of the acquired resistance to copper, it must be necessary to make some cross experiments. From this point of view, the author attempted the following experiment.

Material and Method

The 1st instar larvae of normal flies were transferred to 0.5 mM of $CuSO_4$ containing medium and the normal medium, by twenty individuals to a bottle.

When the pupated flies in these culture bottles began to develop wings, they, were transferred to the tubes of 0.8 cm in diameter and 5 cm in length, one to one, in order to obtain virgin flies. The tube contained 1 ml of PEARL's medium, with a plug of cotton at an end. One hundred and twenty tubes were prepared in one series of experiment. When these pupae became imagos, they were transferred to oviposition tubes to cross according to the design shown in Table 6. The 1st instar larvae (F_1) hatched from these eggs were transferred to the media containing 4 mM of $CuSO_4$, by twenty individuals to a bottle. The emergence rates from these bottles were calculated.

Results

As shown in Table 6, the Cu-strain showed higher emergence rate than the control one as usual. The emergence rates of the F_1 flies of both reciprocal crosses between the control strain and the Cu-strain were even slightly higher than that of the Cu-strain (though the differences were not statistically significant; $0.50 > \alpha > 0.30$). Of course these values were much higher than that of the control strain (the difference was highly significant; $0.001 > \alpha$). Practically, no difference was found out between the flies of both reciprocal crosses.

It seems to be difficult to determine from only these results whether the inheritance of the resistant character to copper was brought about through the

Table 6. Results of the cross test. C: Parent flies which have passed their larval lives in PEARL's medium containing 0.5 mM $CuSO_4$·N: Parent flies which have passed their larval lives in normal PEARL's medium. Figures in parentheses show numbers of bottles used.

P	C♀×C♂			N♀×C♂			C♀×N♂			N♀×N♂		
F_1	CC			NC			CN			NN		
	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%	No. of larvae	Emerged No.	%
1	140(7)	98	70.0	400(20)	281	70.2	400(20)	279	69.7	140(7)	82	58.5
2	200(10)	130	65.0	400(20)	274	68.5	400(20)	282	70.5	200(10)	118	59.0
Total	340(17)	228	67.0	800(40)	555	69.3	800(40)	561	70.1	340(17)	200	58.8

C : Cu-strain
N : Control strain

Examinations of differences in emergence rates among four F_1 generations (χ^2-test).

CC : NC $0.50 > \alpha > 0.30$
CC : CN $0.50 > \alpha > 0.30$
CC : NN $0.05 > \alpha > 0.02$*
NC : CN $0.80 > \alpha > 0.70$
NC : NN $0.001 > \alpha$*
CN : NN $0.001 > \alpha$*

* Significant.

changes of nuclear genes or those of cytoplasmic factors, but there are some possibilities that both nucleus and cytoplasm have worked in evoking these phenomena.

Discussion

In the previous report, the author mentioned that, when a strain of *Drosophila melanogaster* Oregon RS was cultured on the medium containing 0.5 mM of $CuSO_4$, the larvae of the next generation exhibited obvious resistance to copper (YANAGISHIMA and SUZUKI, 1959 a, b). In this paper, the author reported about the changes of the resistant variant in copper resistance and other important physiological characters during the successive culture on the sublethal copper medium or on the normal one. This knowledge will be useful in understanding not only genetic and physiological characters of the change but also the origin of the variant.

Now, the brief review of the present report will be given here. The copper resistance brought about by the short time training on the sublethal copper medium for one generation does not show any change, however long the resistant flies were cultured on the sublethal copper medium successively, so far as the emergence test is done on the medium containing 4 mM of $CuSO_4$. There is no difference in the emergence rate on this test medium between the copper resistant variant cultured on the sublethal copper medium for only one generation and that for twenty generations successively. In the next place, it must be noted that the copper resistance once acquired does not become weak, even when a reverse culture to the normal medium is made. It is interesting, however, that, although the resistance itself does not differ between the copper resistant variant cultured on the sublethal copper medium for only one generation and that for ten generations, the modes of maintenance of the resistance during the reverse culture on the normal medium somewhat differ between the two. Namely, the latter variant shows more frequently statistically significant differences from the control flies than the former, when the tests were executed generation after generation after returning to the normal medium.

As mentioned above, the copper resistance brought about by the short time training on the culture medium containing sublethal dose (0.5 mM) of $CuSO_4$ was transmitted through sexual reproductions. Since the copper medium used for the training was not so toxic that all the sensitive flies were selected away during the training, it is very interesting that the raised resistance could be maintained for many generations even after the transference to the normal medium.

These results show that some genic change into resistance to a given environment may be caused even under the condition where serious selection against sensitive flies can hardly occur, though the mechanism through which the above-mentioned phenomenon takes place has not yet been clear.

Now let us discuss the present results with those of the literature. The problem of the resistance has become an important subject in modern genetics and many investigators have worked in this field, using insects and microbes as materials. Many authors have demonstrated that insects became resistant to various kinds of insecticides. Some of them will be summarized as follows.

In the case of houseflies, two cases have been reported as for the maintenance of resistance after returning to normal condition from such abnormal environments as the addition of insecticides. In one case, an acquired resistance can be kept for a considerable time, and in another case it becomes weaker soon. BRUCE and DECKER (1950) have reported that DDT-resistance produced by training for eighteen generations, can be kept even after the culture in the normal condition (free from DDT) for thirty generations. The same type of conclusions was obtained by MARCH and METCALF (1950), PIMENTEL *et al.* (1950) and HARRISON (1951). MARCH and METCALF (1950) mentioned that a DDT-resistant strain collected in field could keep its resistance for thirty-three generations (about eighteen months) when cultured in a DDT-free condition at their laboratory. On the contrary, HARRISON (1950) demonstrated, using a DDT-resistant strain collected in field, that the resistance decreased to thirty percent of the original one after rearing it in a normal condition for six months.

According to KING (1950), the DDT resistance acquired by selection in a laboratory is apt to become weaker, during the culture in the normal condition. For example, a resistant strain gained by laboratory selection decreased markedly its resistance during the fourteen generations on the normal medium. He also said that a DDT-resistant strain collected in field lost its resistance within a few generations, when cultured on the normal medium free from DDT. Having succeeded in making a DDT-resistant strain of houseflies, which had originally been collected from the fields and selected under a selective pressure of DDT for twenty generations, PIMENTEL *et al.* (1953) returned this strain to the normal condition to observe if there would occur some changes in the DDT-resistance. They reported that the DDT-resistant strain mentioned above lost its resistance completely after having been returned to the normal medium and let it pass there during the twenty-two generations.

There are some reports insisting that the resistance to insecticides in the houseflies increases with the increases of the period during which the flies are subjected to the selection pressure by the insecticides. NEWMAN *et al.* (1949) reported that a strain of houseflies, which had been killed perfectly by exposing to DDT for twenty minutes, became so resistant after the selective training with DDT for ten months that they could live completely even after the exposure of thirty minutes.

In *Drosophila*, BOCHNIG (1954) also demonstrated the gradual increase of resistance to DDT when they were subjected to selective pressure by DDT. In other words, at the start of the experiment, the survival rate was 2.5 per-

cent, but after fourty-five generations it became 65 percent, and at last after seventy-fifth generation it became 98 percent. On the course of this experiment, BOCHNIG released the flies from the DDT-pressure since the fifty-fifth generation and found that the survival rate fell down to 50 percent. It was also stated by him that when the flies were continued to culture under the DDT-pressure for one hundred and forty generations, the resistance to DDT was maintained with great stability even after returned to the normal condition. According to CROW (1954), DDT resistance became three times higher than the original one within six months, four times within a year and six times within three years, when flies were cultured under selective DDT-pressure, the existence of considerable variation in resistance among original natural flies mattering little. KING (1954, 1955 b) and MERRELL and UNDERHILL (1956) reported the same results as CROW's.

In such experiments as described above, the selective pressure of DDT was always forced to apply to adult flies. Some investigators, however, have subjected larvae to the selective pressure of DDT, and have obtained the results as follows. OGAKI and TSUKAMOTO (1953) and TSUKAMOTO and OGAKI (1954) showed that DDT resistance of a strain of *Drosophila melanogaster* increased five times as much as the original one by selecting larvae with DDT for ten generations, but no increase over this was observed by further selection. SOKAL and HUNTER (1954) reported that DDT-resistance could be induced within seventy generations under the selective pressure of DDT at larval stage. KING (1955 b) also obtained the similar results. On the other hand, MELTZER (1956) described that a considerable fluctuation in DDT tolerance took place during the successive culture on the normal medium.

Then, what are the circumstances about the change of resistance to some metallic salts? GREIFF (1943) reported that both productivity and fecundity decreased at first, when a strain of *D. melanogaster* was cultured on $ZnSO_4$ containing medium, but they increased to the normal level after five or six generations. OHSAWA and TSUKUDA (1955) performed an important experiment in connection with the present experiment. They showed that a strain of *D. melanogaster* was made resistant to copper in two generations 1.35 times as much as the original one by selecting resistant flies on a culture medium containing 4 mM of $CuSO_4$. But when they returned these flies to the normal medium after eleven generations on the copper medium the flies lost their resistance, whereas when the returning was performed after fifteen generations, the flies did not lose the resistance. From these experiments they suggested the possibility that something like adaptive variations might sometimes be assimilated into the genetic variation.

Nearly all the results that had recently been obtained were gained, as mentioned above, under the conditions where selection occurred against the more sensitive flies. On the contrary, it must be noticed that the results described in this paper were obtained under the condition that the copper

medium used for the training was not so toxic that all the sensitive flies were selected away during the training. It is very interesting that the copper resistance increases during only one generation by culturing the larvae on a medium containing so little amount of $CuSO_4$ that the larvae could survive in this medium as well as in the normal medium (YANAGISHIMA and SUZUKI, 1959a). This means that the acquired copper resistance is stable enough even on copper free media, i. e., it seems to have some hereditary nature.

It must also be noted that some changes in behaviors and metabolic activities took place associated with the increase of copper resistance and these changes were transmitted in parallel with copper resistance.

The copper resistance is produced within a generation under the conditions where serious selection against sensitive flies can hardly occur and the raised resistance could be maintained for many generations even after the transference to the normal medium. All the results of reciprocal crosses show that the F_1 flies usually exhibit the same resistance to copper as the Cu-strain used as one of the parents.

Now let us take into consideration the literatures on hereditary natures of resistance. Many reports dealing with the cross-experiments of resistant flies to insecticides have been published and most of them show that the inheritance of insecticide resistance follows a simple Mendelian law. TSUKAMOTO and OGAKI (1953) described that the DDT-tolerance in *D. melanogaster* depends upon the dominant gene or genes located on chromosome II, and the gene or genes of DDT-tolerance rules BHC-tolerance at the same time. KIKKAWA (1953), TSUKAMOTO (1955) and TSUKAMOTO and HIROYOSHI (1956) described that the gene or genes of DDT-tolerance on chromosome II shows cross-resistance to nicotine. HARRISON (1951, 1954) has shown the same conclusion in the experiments with houseflies. She mentioned that DDT-tolerance depends on a single recessive gene. There are a considerable number of reports which show that the DDT-tolerance in *D. melanogaster* is a semidominant character whose origin is polygenic and its inheritance shows neither sex-linkage nor maternal effects. The same conclusion was reached by BUSVINE and KHAN (1955), using houseflies and BHC. In these cases, the F_1 flies between tolerant and susceptible flies show approximately intermediate tolerance between both parents, regardless of reciprocal crosses (KING, 1954, with *D. melanogaster* and DDT).

Then, we can find some reports showing the role to be played by copper as a mutagene. LAW (1938) and MAGRZHIKOVSKAYA (1938) have shown that copper induced mutation at X-chromosome in *D. melanogaster*. DEMEREC *et al.* (1951) demonstrated mutagenic action of copper, using a streptomycin dependent mutant of *Escherichia coli* as material. It will not be important to think in the present case, however, that copper can act on flies as a mutagene, but it is hard to attribute the acquisition of the copper resistance to mutagenic action of copper only, for the copper resistance increased by rearing in the sublethal copper medium.

In the next place, some discussions will be made on the assimilation of temporary adaptive characters into genetic ones. There have been many investigators who claimed that a main cause of heritable variation is found in some changes of environmental factors, among whom DARWIN may be mentioned. He published "The variation of animals and plants under domestication" in 1868, and emphasized this point. According to him, if it were possible to expose all the individuals of a species during many generations to absolutely uniform conditions of life, there would be no variability. Therefore, the variability depended mainly on the changed conditions of life, and it was mediated by use and disuse of orgrans.

On the other hand, the author wishes to cite here STERN's opinion as a representative one of Neo-Darwinists. He has mentioned in his "Variation and hereditary transmission" (1959) that Neo-Darwinism believes that there is no direct connection between changes in organs brought about through use and disuse and mutations in genotype, and this conclusion is considered to have been led from many experimental results. He has also described that the genetic variations which developed under a specific environment grant much more new characters to the organism than it really requires in current life, and has called this phenomenon as "Bonus principle".

Thus STERN has a firm belief that adaptations in the history of evolution might have been brought about through the combination of random mutations and selections. However, there are some other investigators who suppose adaptive processes can be assimilated in some cases into genetic characters, and as the representative of this kind of investigators, we can quote WADDINGTON (1953 a, b, 1959). He proposed the theory of "genetic assimilation", the idea of which is as follows. Tissues and organs in multi-cellular organisms have an obvious tendency to develop into normal adult through a definite "canal", even under an abnormal environment, and he called this phenomenon as the canalization of development. Some kinds of adaptive variations, which are induced only when some environmental changes are operated upon that organisms, gradually acquire genetic natures if those environmental changes are made to operate repeatedly, and at last, these changed characters become to appear normally in the course of development even under the normal environment. He demonstrated that crossveinless caused only by heat shock at first became to occur spontaneously in a larger part of *Drosophila* flies without heat shock when heat shock had been operated repeatedly for several generations. This idea is very much similar to SCHMALHAUSEN's "stabilizing selection" (1949).

TANAKA (1958) employed the polygene theory to explain WADDINGTON's result, and as the natural result, he denied the opinion that the adaptive process, other than as the result of natural selection, was partaken in the process. This opinion is the same one as that of STERN's (1959) and may also be the representative one of the current New-Mendelists. But as mentioned repeatedly, the author has been led through many experiments and also by

some literatures to the conclusion somewhat different.

Though many reports concerning with changes into resistance to a given environment or variation caused by a given environment have been published as mentioned partly above, we cannot offer any conclusive remarks which throw some light on the mechanism of the changes dealt with in the present paper from them.

Nevertheless, a series of works performed by the present author is worthy to note, for it shows the possibility that genic changes can take place under moderate conditions in a short time.

Summary

The author has already reported that when a strain of *Drosophila melanogaster* Oregon RS was cultured on 0.5 mM copper containing medium for one generation, the flies of the next generation showed remarkable resistance to copper. In this report, the changes in the copper resistance and some other characters evoked with increase of copper resistance were studied, rearing copper resistant flies on the copper containing medium or on the normal one for many generations. The main results are as follows.

1. There was no difference in emergence rate on 4 mM copper-containing medium between the flies cultured on 0.5 mM copper medium for twenty generations and those for only one generation.

2. The copper resistance acquired through rearing on 0.5 mM copper medium could be maintained during reverse culture on normal medium for many generations, but the longer the flies were trained on the copper medium, the more completely the resistance was maintained.

3. The behavior change found in the copper resistant flies also did not change during culture for successive generations, both on copper medium and normal one.

4. Copper resistant flies were more sensitive to some enzyme inhibitors than normal flies as mentioned previously, and this character also did not change for many generations on both copper medium and normal one.

5. The cross experiments showed that the raised copper resistance in the Cu-strain was inheritable and F_1 of the reciprocal crosses was always as tolerant as the Cu-strain used as parents.

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