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Study of the Effects of Sodium Chloride on Segregation-Distorter Action in *D. melanogaster* 1. Treatment of Sodium Chloride on Whole Developmental Stages.

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초파리의 SD 작용에 미치는 Sodium Chloride의 영향에 대하여
그 발생전 시기에 있어서의 처리

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적 요

초파리의 4가지 계통을 그 발생전 시기를 통하여 NaCl 배지에서 사육하였을때 우화율과 SD 작용의 변동을 조사 검토하기 위하여 6가지 농도의 NaCl 사육배지를 만들어 실험한 결과는 다래와 같다.

1. 우화율이 SD의 4계통간에는 유의한 차가 없으나 NaCl 농도에 따르는 차이는 매우 유의하다.
2. 우화율은 NaCl 농도가 커질수록 낮아지는 경향이 있으며 0.0M(표준배지)에서 0.3M 농도까지는 NaCl에 대한 저항성이 강한 편이나 0.5M 이상의 농도에서는 저항성이 급격히 약해진다.
3. 1.0M 이상의 NaCl 농도에 대해서는 알이 전혀 부화하지 못하는데 이것은 1.0M 이상의 NaCl 농도에 대한 저항성은 전연 없다고 볼수 있다.
4. SD의 작용 즉 k 값은 SD 계통간이나 NaCl 농도간이나 다 같이 유의한 차를 본수 없는데 이것은 NaCl 배지이더라도 일단 우화만하면 SD작용에는 변동이 없음을 말해 주는 것이다.

INTRODUCTION

Kataoka(1967) analyzed a system of suppressor for the SD action in a natural population of *Drosophila melanogaster* from Japan and found that most of the suppressors were located on the X chromosome. Chung and Kang(1969 a and b) reported that the suppressor system was also involved in natural populations of *Drosophila melanogaster* from Korea and the location of the system was found to be in the X chromosome.

In order to see an effect of temperature on the SD action, Mange(1968) treated SD chromosome at various temperatures (30°, 28-29°, and 19°C) for varying lengths of time(1 to 12 days) at various stages of development (larva-adult) and the results of his experiments showed that not all SD chromosomes were equally sensitive to temperature, the most sensitive period occurred around the time of early meiosis, temperature sensitivity of SD depended on the stage of meiosis of a particular sperm rather than the developmental stage of the individual fly, and the temperature effect was found not to be heritable.

Chung and Kang(1968) investigated the effects of constant and non-constant temperatures on the SD action in *Drosophila melanogaster* and found that the sensitivity of SD showed no significant differe-

nce between constant and non-constant temperature. Miyoshi(1961) performed a series of experiments on the resistibility and the requirement of Drosophila for sodium chloride (NaCl) from the genetical and physiological stand point and he examined the strain differences of *Drosophila melanogaster* in the resistibility and the heritability of this character, and the results showed that the resistibility to NaCl varied in different strains; the *bw* Kyoto strain exhibited a strikingly high resistibility, and there was correlation between resistance and the mutant markers or lack of markers in the various strains, and the resistibility to NaCl was found to be hereditary.

Miyoshi and Nakamura(1968) examined how NaCl of high concentrations in the medium affected the developmental process in both the resistant strains and the susceptible ones, and the results showed that (1) eggs of both the resistant and the susceptible strains were quite insensitive to NaCl in the medium unless they were dechorionated, (2) larvae of *Drosophila melanogaster* were more sensitive to high concentration of NaCl at the first instar larval stage than succeeding stages, the viability of the second instar larvae was less affected by the NaCl medium that of the first instar larvae, and in the third larval stage, even NaCl susceptible strains were hardly affected by high concentrations of NaCl, and (3)flies were not fatally affected by the NaCl medium at the pupal stage, but the time for completion of the pupal stage was markedly prolonged.

The present paper deals with the results of the experiments, in which the resistibility to NaCl in whole developmental stages is investigated by emergence rate and effects of NaCl on the SD action in *Drosophila melanogaster* were examined.

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MATERIALS AND METHODS

The following stocks were used in the present experiments:

1. Original-SD chromosome strains: SD-72[SD, Ac (SD, St(SD)] and SD^{NH}-2[SD, Ac(SD), St(SD)] were originally collected from a natural population in Medision, Wisconsin, United States and in Odate, Japan, respectively. These orignal-SD chromosomes carry St(SD), so that the SD action is stable and is invariably strong (k values are usually more than 0.90).

2. Recombinant-SD chromosome strains: R-1[SD, Ac(SD),-*bw*] and R(SD^{NH}-1)-1 [SD,Ac(SD),-*bw*] were originally obtained as recombinants from the original-SD/*cn bw*. Since these strains had lost St (SD) by recombination, the SD action is rather unstable and the k value is somewhat reduced(k=0.80 or more).

Both of these SD strains have been kept by backcrossing to the standard *cn bw* females for many generations, therefore, their genetic background is that of standard *cn bw* strains.

3. Standard stock: *cn bw* laboratory stock is marked by cinnabar and brown on chromosome 2 and the phenotype is white eyes. These stocks have been reared continuously by small mass-culture for many years in the genetic laboratory of Department of Science Eduation, Ewha Womans University.

Stock of SD and *cn bw* have been raised continuously at 25±1°C in the constant temperature room on the culture medium of a standard corn meal, yeast, agar-type with a few drops of propionic acid as a mold inhibitor.

In order to minimize the NaCl content in the medium, the following formula was used as the standard medium: agar powder 2g, sugar 10g, dry yeast 10g, tartaric acid 0.4g, propionic acid 2-3 drops, and deionized water 1000cc; propionic acid was used to inhibit the growth of molds; tartaric

acid was added to the agar solution at temperatures below 70°C to avoid hydrolysis of agar; yeast was killed by boiling.

The present experiments were performed at 25± 1°C, and the NaCl medium was prepared by adding NaCl at a concentration of 1.0M, 0.7M, 0.5M, 0.3 M, 0.1M, and 0.0M to the standard medium. A concentration of 0.0M NaCl was prepared for the control experiment.

RESULTS AND DISCUSSION

Eggs from populations of ten pairs of flies of each SD strain were transferred to the culture medium three to four hours after being laid. Groups of 50 eggs each were put on a slide glass(2.5 cm wide and 7.5 cm long), which had been covered with 5 ~6g of either the NaCl medium or the standard medium. The glass plate was introduced into a vial 3 cm in diameter and 12 cm in length. A piece of moist filter paper 2 cm in width and 10 cm in length was provided in each vial to give suitable moisture and the vial was closed with cotton plug and kept at 25±1°C in the constant temperature room.

Ten vials, each of which contained 50 eggs(thus total of 500 eggs) were prepared for each concentration of NaCl in each SD strain so that total of 200 vials(10,000 egg) were set up in the present experiments.

Under the experimental conditions described above, the mumber of emerging adults was counted and the emergence rate was calculated for each vial and averaged for each concentration of NaCl in each SD strain. The results were shown in Table 1 and Figure 1. No eggs were hatched from the culture medium containing a concentration of 1.0M NaCl so that the result of it is excluded in Table 1 and Figure 1.

As illustrated in Table 1 and Figure 1, the emergence rate is not significantly different among strains but strikingly different among concentrations of NaCl. The results of analysis of variance also indicated no significant difference of emergence rate among stains($F=0.62$, $n_1=3$, $n_2=12$, $p>0.05$) and highly heterogeneous emergence rate among concentrations of NaCl) $F=63.90$, $n_1=4$, $n_2=12$, $p<0.01$). The emergence rate is found to be almost homogeneous among the concentration of NaCl, 0.0M, 0.1 M, and 0.3M but the rate drops strikingly to 30—40% at 0.5M of NaCl and then to 10—20% at 0.7M of NaCl. This suggests the four SD strains are considerably resistible to the NaCl from a concentration of 0.0M to 0.3M but are susceptible from 0.5M or higher concentrations of NaCl. It is somewhat peculiar, as seen in Table 1 and Figure 1, that the emergence rates of three SD strains at 0.0M (control media) was lower than at 0.1M against expectation of higher emergence rate on the standard culture media than on the media containing 0.1M of NaCl. This could be due to the matter of chance. In fact, statistical analysis shows no significant difference

Table 1. Emergence rates(%) in the four SD strains of *Drosophila melanogaster* in the culture media containing various concentrations of NaCl

Strain	Conc. of NaCl				
	0.0M	0.1M	0.3M	0.5M	0.7M
SD-72	81.0	84.9	71.0	32.4	12.0
SD^{NH}-2	83.6	88.6	83.0	43.6	15.3
R- 1	78.1	79.6	67.2	40.8	10.0
R(SD^{NH}-1)-1	81.8	80.8	81.4	31.2	18.0

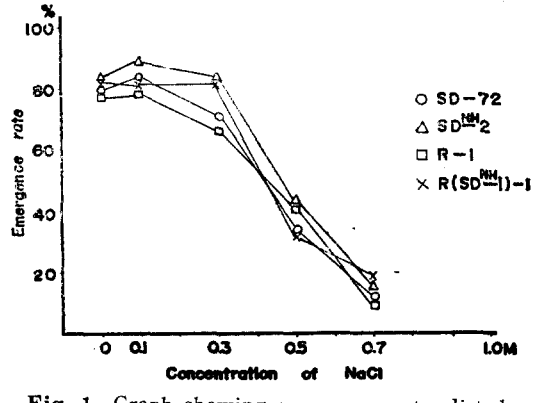

Fig. 1. Graph showing emergence rates listed in Table 1.

between these concentrations.

Miyoshi(1961) reported the results of the experiments on the strain difference of *D. melanogaster* in the resistibility to the 1.0M NaCl media by examining the emergence rates of 12 mutants and five wild-type strains. The results showed that the resistibility to NaCl (emergence rate) varied in different strains; one of the strains (*bw* Kyoto) exhibited a striking contrast to other strains by its high resistibility in which about one half of the eggs grew to imagoes in the medium containing 1.0M NaCl. As far as the four SD strains are concerned, neither strain difference in the energence rate on the NaCl media nor remarkably resistible strains to NaCl are found in the present experiments. The most strains used in the experiment of Miyoshi(1961) exhibited high resistibility to 1.0M NaCl, some strains showed a considerable resistibility to even 1.3M of NaCl. On the other hand, no eggs of all four SD strains in present experiments are hatched on the media containing 1.0M NaCl. This striking contrast may be explained by some possibilities one of which might be the different formula of the food media from that of Miyoshi(1961). The formula used in the present experiments is similar to that of Miyoshi except that a few drops of propionic acid is used as a mold inhibitor instead of n-butyl benzoate in Miyoshi's formula. This matter must be made clear in the further experiments.

Miyoshi(1961) also examined the emergence rates of the three strains selected from 17 ones on the culture media containing various concentrations of NaCl (0.0M, 0.1M, 0.3M, 0.5M, 0.7M, 1.0M, and 1.3M) and the results showed that the emergence rate of two of three strains decreased as the concentrations of NaCl increased. This is roughly agreed with the results of the present experiments.

In order to examine the effects of NaCl on the SD action ten males (heterozygous for the SD and the *cn bw* chromosome) were sampled from each vial after calculation of emergence rate, and were individually mated to two or three homozygous *cn bw* virgin females. And the *k* values, the proportion of the SD chromosomes recovered among progeny flies of the mating, were calculated and averaged for each concentration of NaCl in the four SD strains. The results are shown in Table 2 and in Figure 2.

Table 2. The *k* values obtained from mating the heterozygous SD males emerged on the media containing various concentrations of sodium chloride, to *cn bw* females

Strain	Conc. of NaCl				
	0.0M	0.1M	0.3M	0.5M	0.7M
SD-72	0.998	0.996	0.992	0.999	0.995
SD^{NH}-2	0.982	0.927	0.956	0.958	0.972
R- 1	0.865	0.920	0.840	0.846	0.898
R(SD^{NH}-1)-1	0.907	0.963	0.967	0.923	0.951

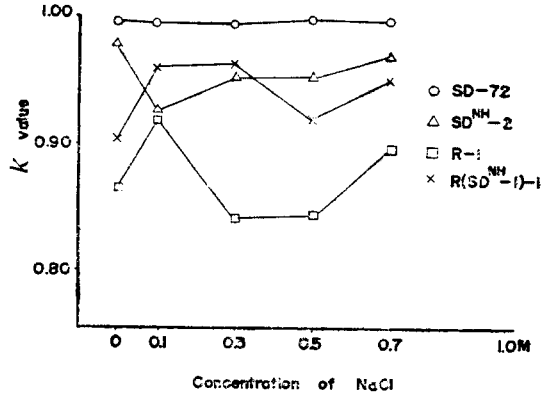

Fig. 2. Graph showing the *k* values listed in Table 2.

As seen in Table 2 and Figure 2, the difference in *k* values is not significant among strains and also among concentrations of NaCl. Analysis of variance also shows the same results ($F=3.00$, $n_1=3$, $n_2=12$, $p>0.05$ for the strains; $F=0.10$, $n_1=4$, $n_2=12$, $p>0.05$ for the concentrations of NaCl). Thus the effects of NaCl on the SD action are found to be not significant. It is concluded that the SD action is not affected as far as once emerged from the culture media whether containing NaCl or not.

The *k* values in the strains, SD-72 and SD^{NH}-2 are

higher than in the remaining strains, R-1 and R (SD^{NH}-1)-1, even though the difference of k among strains is not statistically significant, as mentioned above. This is rather expected results as Chung pointed out(1968). Since the recombinant-SD strains, R-1 and R(SD^{NH}-1)-1 had lost St(SD) by recombination, the SD action is rather unstable and the k value is somewhat reduced. Thus the k values in these recombinant strains must be lower than in the original-SD strains, SD-72, and SD^{NH}-2 in which St (SD) is maintained.

SUMMARY

The present paper deals with the results of the experiments, in which the resistibility to NaCl in whole develomental stages is examined by the emergence rate and the effects of NaCl on the SD action in *Drosophila melanogaster*. The four SD strains and one mutant strain(*cn bw*) are used and NaCl media are prepared by adding NaCl at a concentration of 1.0M, 0.7M, 0.5M, 0.3M, 0.1M, and 0.0M to the standard media for the present investigations. The results are given below.

1. The emergence rate (resistibility to NaCl) is not significantly different among strains but strikingly different among concentrations of NaCl.

2. The emergence rate decreases as the concentration of NaCl increases; the four SD strains are considerably resistible to the NaCl from a concentration of 0.0M to 0.3M but are susceptible from 0.5M or higher concentrations of NaCl.

3. No eggs are hatched from the culture media containing a concentration of 1.0M NaCl. This suggests that the SD strains are not resistible to NaCl at a concentrations of 1.0M or higher.

4. The difference in k values is not signficant among strains and also among concentrations of NaCl. Thus the SD action is not affected as far as once emerged from the culture media whether containing NaCl or not.

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