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Study of the Effects of Sodium Chloride on Segregation-Distorter Action in *D. melanogaster*
4. Treatment of Sodium Chloride on the Pupal Stages

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초파리의 SD 작용에 미치는 Sodium Chloride의 영향에 대하여
4. 번데기 시기에 있어서의 처리

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적 요

초파리의 번데기 시기에 NaCl을 처리하였을 때 우화율과 SD 작용에 변동이 있는가를 보기 위하여 6가지 계통, 즉 SD 계통 4가지와 다른계통 2가지를 써서 6가지 농도의 NaCl 사육배지를 만들어 실험한 결과는 다음과 같다.

1. 우화율(즉 NaCl에 대한 저항성)은 계통간이나 NaCl의 농도간이나 다같이 매우 유의적인 차이를 보이고 있다.

2. 우화율은 NaCl 농도가 높을수록 낮아지는 경향이 있으며, 이 저하는 완만한 편이고 발생의 각 단계중에서 번데기 시기가 NaCl의 영향을 가장 적게 받는다.

3. SD 계통의 우화율은 다른 계통에 비하여 대체로 낮은 편인데, 이것은 SD 계통이 NaCl에 대한 저항성이 다른 계통에 비하여 약한 것을 말한다.

4. SD 작용의 척도라고 볼 수 있는 k 값은 NaCl의 농도간에 유의한 차가 없으나 계통간에는 유의적인 차이를 보이고 있으며, Original-SD 계통의 k 값은 Recombinant-SD 계통의 k 값보다 높고 또한 안정성을 나타내고 있다.

5. NaCl은 SD 작용에 영향을 미치지 못하며 발생의 어느 단계에 NaCl을 처리하거나 관계없이 일단 우화만 되면 SD 작용에는 변동이 없다.

INTRODUCTION

The extensive investigations on the phenomenon of segregation-distortion(SD) of *Drosophila melanogaster* have been carried out by many investigators since it was first found. A system of suppressor for the SD action in a natural population of *D. melanogaster* was analyzed by Kataoka

(1967), Hihara(1968), Chung & Kang (1969 a & b). An effect of temperature on the SD action was also studied by Mange (1968), Chung & Kang (1968), and Hihara (1970).

A series of experiments on the resistibility and the requirement of Drosophila for sodium chloride (NaCl) was performed from the genetical and physiological standpoint by Miyoshi (1961) and Miyoshi & Nakamura (1968).

Kang *et al.*(1970) reported the results of the experiments, in which the resistibility to NaCl in whole developmental stages was investigated by examining the emergence rate and the effects of NaCl on the SD action in *D. melanogaster* were analyzed. They used the four SD strains and one mutant strain (*cn bw*) and NaCl media were 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. The results showed that the emergence rate was not significantly different among strains but strikingly different among concentrations of NaCl and the SD action was not affected as far as once emerged from the culture media whether containing NaCl or not.

Chung & Kang (1971) treated the various concentrations of NaCl at the second and the third instar larval stage and examined the effects of NaCl to the emergence rate and the SD action. Their results were found to be roughly agreed with those of Kang *et al.* (1970) except that larvae of *D. melanogaster* were more sensitive to NaCl media at the second instar larval stage than the third one and that the difference in k values was not significant among concentrations of NaCl and among strains when treated NaCl to the second instar larval stage but highly heterogeneous among the strains when treated NaCl to the third instar larval stage.

In the above investigation, the treatment of NaCl at the second and the third instar larval stage was concerned but the treatment of it at the first instar larval stage was excluded. Kang (1971) examined the effects of sodium chloride on SD action in *D. melanogaster* when treated it at the first instar larval stage. He used the four SD strains and two other mutant strains (*cn bw* and Oregon-R) and prepared NaCl media by adding NaCl at a concentration of 0.0M, 0.1M, 0.3M, 0.5M, 0.7M, and 1.0M to the standard media for his experiment and obtained the results which were roughly similar to those of the previous works (Kang *et al.* 1970, Chang & Kang 1971, and Kang 1971).

The present experiment was designed to see if NaCl affected the SD action of *D. melanogaster* when treated it at the pupal stage.

MATERIALS AND METHODS

The following six strains were used in the present work:

1. *cn bw*: standard laboratory mutant stock of *D. melanogaster* carrying cinnabar and brown eye color recessive mutants. This strain shows white colored eyes.

2. Oregon-R: standard laboratory wild stock.

3. 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 Madison, Wisconsin, United States and in Odate, Japan, respectively. These chromosomes

carry St(SD) so that the SD action is stable and is invariably strong.

4. 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*. The SD action of these strains is rather unstable and the *k* value is somewhat reduced since these strains had lost the element, St (SD) by recombination.

Oregon-R, *cn bw* and stocks of SD 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. The following formula of the food media used in the present experiment was adopted as the standard media in order to minimize the NaCl content in the media: agar 2g, sugar 10g, dry yeast 10g, tartaric acid 0.4g, n-butyl benzoate 1ml, and deionized water 1,000ml; tartaric acid was added to the agar solution at temperature 70°C to avoid hydrolysis of agar; n-butyl benzoate was used to inhibit the growth of molds; yeast was killed by boiling.

The experiment was done at 25±1°C and the NaCl medium was prepared by adding NaCl at a concentration of 1.0M, 0.7M, 0.5M, 0.3M, 0.1M, and 0.0M (as control) to the standard medium.

RESULTS AND DISCUSSION

Young pupae which passed the egg and larval stage in the standard media and attached on the wall of the vials were collected and transferred to the NaCl media and kept at 25±1°C in the constant temperature room.

As in the previous works (Kang *et al.* 1970, Chung & Kang 1971, Kang 1971) ten vials, to each of which transferred 50 pupae (thus total of 500 pupae) were prepared for each concentration of NaCl in each strain so that total of 3,600 vials (total of 18,000 pupae) were set up. The number of emerging adults was counted and the emergence rate was calculated for each vial and averaged for each concentration of NaCl in each strain. The results were shown in Table 1 and Figure 1.

Ten males, heterozygous for the SD and the *cn bw* chromosome were sampled from each vial calculation of emergence rate, and were individually mated to two homozygous *cn bw* virgin females and the *k* values were calculated and averaged for each concentration of NaCl in the six strains. The results were presented in Table 2 and Figure 2.

The results of the statistical analysis indicated that very significantly different emergence rate was shown not only among the strains but also among concentrations of NaCl ($F=5.09$, $n_1=5$, $n_2=25$, $p<0.01$ among strains; $F=20.63$, $n_1=5$, $n_2=25$, $p<0.01$ among concentrations of NaCl).

No significant difference of emergence rate was shown among the four SD strains and the emergence rates of *cn bw* and Oregon-R showed higher values than of the above four SD strains. This tendency is agreed with the results of Kang's experiment(1971).

As seen in Figure 1, the emergence rate decreases as the concentration of NaCl increases but the rate of decrease shows

Table 1. Emergence rate(%) in the six strains of *D. melanogaster* when treated various concentrations of NaCl to the pupal stage

Strain	No. of pupae tested	Concentration of NaCl					
		0.0M	0.1M	0.3M	0.5M	0.7M	1.0M
SD−72	500	91.6 (458)	87.4 (437)	90.2 (451)	71.4 (357)	70.8 (354)	68.8 (344)
SD^{NH}−2	500	93.2 (466)	92.0 (460)	85.4 (427)	74.8 (374)	72.6 (363)	67.2 (336)
R−1	500	88.2 (441)	85.6 (428)	84.6 (423)	77.0 (385)	74.8 (374)	72.0 (360)
R(SD^{NH}−1)−1	500	93.0 (465)	91.8 (459)	79.2 (396)	77.6 (388)	76.8 (384)	71.4 (357)
cn bw	500	91.0 (455)	89.0 (445)	82.4 (412)	82.2 (411)	81.6 (408)	80.6 (403)
Ore−R	500	94.4 (472)	94.2 (471)	87.8 (439)	86.0 (430)	89.6 (448)	84.6 (423)

Nos. in ( ) indicate the number of flies emerged.

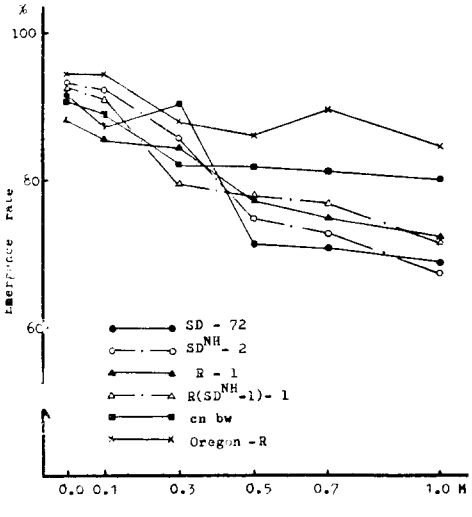

Fig. 1. Graph showing emergence rates listed in Table 1.

rather constant as the concentrations of NaCl increases so that the curves presented in Figure 1 are not steep and decrease of the emergence rates *cn bw* and Oregon-R is not striking compared to the four SD strains. This implies that *cn bw* and Oregon-R strains are more resistible to the NaCl than the four SD strains. It is noticeable that not a few flies are emerged from the media of 1.0M of NaCl.

The decrease of the emergence rate was very striking (so that the curves were very steep) and very rare flies were emerged from the media of 1.0M of NaCl when treated NaCl at the first larval stage (Kang 1971), at the second and the third instar larval stage (Chung & Kang 1971). These are disagreed with the results of the present experiment as mentioned above. Thus a conclusion can be established such that the effect of NaCl to the emergence rate when treated the NaCl at the pupal stage of *D. melanogaster* is not significant even though the emergence rate decreases as the concentration of NaCl increases.

As shown in Table 2 and Figure 2, the difference in *k* values is significant among the four SD strains but not among concen-

trations of NaCl. Analysis of variance also indicates significant difference in k values among strains ($F=17.23$, $n_1=3$, $n_2=15$, $p<0.01$) but not among concentration of NaCl ($F=0.01$, $n_1=5$, $n_2=15$, $p>0.05$). Thus the effects of 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. These phenomena are agreed with the results of experiments of Kang *et al.* (1970), Chung & Kang (1971), and Kang (1971).

As seen in Figure 2, the k values in the two original SD stains, SD-72 and SD^{NH}-2 are higher than in the two recombinant SD strains, R-1 and R(SD^{NH}-1)-1 and the range of variation of k values in the recombinant SD strains is wider than that of the original SD ones. This is rather expected phenomenon as the previous investigators pointed out (Chung & Kang 1968, Kang *et al.* 1970, Chung & Kang 1971, and Kang 1971). Since the recombinant SD strains had lost St(SD) by recombination, the SD action is rather unstable and k values is somewhat reduced. Thus k values in these strains must be lower than in the original SD strains in which St(SD) is maintained.

Thus the effects of NaCl on the SD action are not significant regardless of the treatment of NaCl at any developmental stage of *D. melanogaster*.

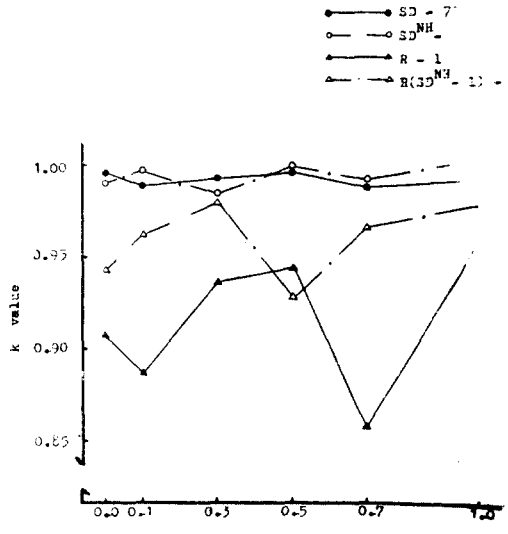

Fig. 2. Graph showing the k values listed in Table 2.

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

Strain	Concentration of NaCl					
	0.0M	0.1M	0.3M	0.5M	0.7M	1.0M
SD−72	0.994	0.989	0.992	0.993	0.988	0.991
SD^{NH}−2	0.990	0.996	0.984	0.997	0.991	0.998
R−1	0.909	0.886	0.937	0.944	0.857	0.956
R(SD^{NH}−1)−1	0.945	0.962	0.980	0.928	0.966	0.976

SUMMARY

In order to see if NaCl affected the the emergence rate and the SD action when treated it at the pupal stage of *Drosophila melanogaster*, an experiment was performed

in which the resistibility to NaCl at the pupal stage was examined by the emergence rate and the effects of NaCl on the SD action was studied by *k* values. The four SD strains and two other strains (*cn bw* and Oregon-R) were used and NaCl media were prepared by adding NaCl at a concentration of 0.0M, 0.1M, 0.3M, 0.5M, 0.7M, and 1.0M to the standard media for the present experiment. The following conclusions were established:

1. The emergence rate (resistibility to NaCl) when treated NaCl at the pupal stage is significantly different not only among the strains but also among concentrations of NaCl.

2. The emergence rate decreases as concentration of NaCl increases but the rate of decrease shows rather constant so that the curves of decrease are not steep.

3. The emergence rate of the *cn bw* and Oregon-R strains shows higher value than of the four SD strains, implying that the resistibility to the NaCl is lower in the SD strains than in the two strains; not a few flies are emerged from the media of 1.0M of NaCl.

4. The difference in *k* values is not significant among concentrations of NaCl but among strains, the *k* values of the original SD strains are rather higher and constant but those of the recombinant SD strains are rather low and unstable.

5. The SD action is not affected by NaCl as far as once emerged from the culture media whether containing NaCl or not in any developmental stage. Thus such an experiment on NaCl effect is of little value to analyze the mechanism of the SD action in *D. melanogaster*.

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