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A Study of Alcohol Dehydrogenase of *Drosophila melanogaster* in Korea by Starch Gel Electrophoresis

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Starch gel 전기영동법에 의한 한국산 초파리의 alcohol dehydrogenase (ADH)에 관한 연구*

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摘 要

한국산 초파리(*Drosophila melanogaster*)의 starch gel 영동상을 조사하기 위하여 춘천, 진주, 신촌(서울), 대전의 초파리 4계통을 starch gel media를 사용하여 벤드의 분포, 염색강도 및 이동도를 비교 검토한 결과는 다음과 같다.

1) 춘천, 진주, 신촌(서울)의 3계통은 벤드의 수와 분포, 염색강도, 이동도가 다 같이 심한 변이를 나타내고 있는데 반하여 대전계통은 변이가 거의 없다.

2) 이러한 전기영동상의 차이는 대전 계통의 초파리 집단이 ADH 구성에 관하여 동질적(homogeneous)이고 다른 3계통의 집단은 이질적(heterogeneous)이라는 것을 암시하고 있다.

3) 전기영동상이 4계통의 초파리에서 다 같이 암·수에 따라 차이를 보이고 있는데 이것은 설명하기 매우 어려운 문제로서 장차 분석 검토할 필요가 있다고 본다.

4) Starch gel media가 cellulose acetate media 보다 해상력이 월등하다는 것이 재확인 되고 있다.

5) Agar gel이나 acrylamide gel 같은 다른 영동 media를 써서 ADH의 전기영동 실험을 하여 starch gel media를 사용한 본 실험의 결과와 비교하는 것과 ADH에 관한 유전적 분석이 시급히 요구되는 앞으로의 과제이다.

INTRODUCTION

In recent years a large number of naturally occurring protein polymorphism have been described in different animal groups. This development is largely due to improvements in the techniques for protein separation, such as the method of

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starch gel electrophoresis (Smithies 1955). The combination of starch gel electrophoresis with different enzyme staining methods has also made it possible to study with greater efficiency electrophoretic variations in various enzymes (Hunter & Markert 1957). The concept of an isozyme variants have been described in a large variety of organisms ranging from protozoa to man (cf. Brewer 1970).

Studies on the isozymes of insects are specially promising because their exceptionally good experimental materials for the investigation of isozymes, particularly from a genetic point of view. The dramatic changes occurring during the developmental life history of insects lead to great changes in enzymatic composition at successive stage in development. For this reason, a number of insect geneticists have begun to look for isozymic forms in insects, particularly house fly (Ogita 1962, 1964, 1968; Ogita and Kasai 1964, 1965 a,b,c) and drosophilid fly (cf. Chung & Lee 1972).

Several reports have appeared recently on multiple forms of alcohol dehydrogenase (ADH) in the drosophilid species: *D. subobscura, D. pseudoobscura, D. simulans, D. alpina, D. bifaciata, D. silvestris, D. obscura, D. ambigua* (Lakovaara & Saura 1970, Saura & Lakovaara 1971); *D. melanogaster* (Johnson & Denniston 1964, Grell *et al* 1965, Ursprung & Leone 1965, Rasmuson *et al* 1966, Courtright *et al* 1966, Jacobson 1968, Ursprung & Carlin 1968, Dunn *et al* 1969, Bremer *et al* 1971).

In order to establish a system of biochemical population genetics in the Korean natural populations of *Drosophila melanogaster*, Chung and Lee (1972) examined the electrophoretic patterns, mobilities, and activities of the ADH isozymes in the strains of 18 localities of Korea (Jeju, Kuje, Namhai, Pusan, Taiku, Kimchun, Yusu, Kunsan, Mokpo, Youngkwang, Kwangju-Chunnan, Junju, Taijun, Chungju, Jeungpyung, Chunchun, Mapo-Seoul, and Sinchon-Seoul) with cellulose acetate media and obtained the results: 1) The eletrophoretic patterns of the ADH isozymes of *D. melanogaster* was observed to be different each other among the strains from 18 localities of Korea, the strains from the three localities (Chungju, Jeungpyung, and Taijun) showed three bands, the Junju strain showed two bands and the remaining strains from 14 localities exhibited only one band in the zymograms. 2) The electrophoretic mobility was slower in the five strains (Mokpo, Yusu, Pusan Chunchun and Kwangju), faster in the three strains (Taiku, Taijun, and Jeungpyung), and intermediate between them in the remaining strains from ten localities. 3) The activity of the ADH isozyme was extremely strong in the Taiku strain. and 4) Those results implied that the genetic constitution of the ADH isozymes was different each other among the strains from various localities of Korea.

Through the use of the starch gel zymogram technique because of its resolving

power superior to any other type of zone electrophoresis, a wide variety of electrophoretic enzyme variations have been described in recent years (Johnson & Sakai 1964). The purpose of the present investigation is to examine the electrophoretic patterns of the ADH isozymes in the strains of *Drosophila melanogaster* from the four localities of Korea by using starch gel media instead of cellulose acetate strip used in the previous work (Chung & Lee 1972).

MATERIALS AND METHODS

1. Collection: The collections were made from the three localities of Korea (Chunchun, Jinju, and Sinchon-Seoul) during June-September 1972. Flies were captured from breweries and traps with fermentating peaches, apples, or grapes. Flies thus collected were kept in the constant temperatur room (25±1°C) until they were used in the experiment. In addition to the above three strains, one more strain, the Daijun strain, was used which had been raised for about 15 months since captured originally in the Daijun area of Korea. Thus the four strains of *Drosophila melanogaster* were used in the present experiment.

The food used was a standard corn meal, yeast, agar type with 0.5% propionic acid as a mold inhibitor.

Approximately 5-10 generations (except the Daijun strain) had elapsed between collection and examination for the alcohol dehydrogenase.

2. Electrophoresis: The starch gel electrophoresis was carried out using the discontinuous system of buffers (Poulik 1957). 24 flies (12 males and 12 felmales) were sampled from each strain and single adult flies were placed on a microscope slide and homogenized with a lancet in a small drop (approximately 0.02ml) of distilled water. The homogenate was absorbed on to a 5×9mm piece of filter paper (Toyo No. 50) and inserted into a vertical slot in the starch gel. The samples were electrophoresed for about three hours at a voltage gradient of 5 V/cm for the first 10 minutes and thereafter 15V/cm at 7°C in the constant temperature room.

After electrophoresis the gels were sliced horizontally and stained for the ADH activity in the following solution: 100ml. *tris*hydroxymethylaminomethan-hydrochlorde buffer, pH 8.5, 0.1M 5 ml. 95% ethanol; 25mg diphosphopyridine nucleotide; 25mg *p*-nitroblue terazolium; 7 drops 0.5% methylene blue. Staining was complete in about four hours at 37°C in incubator and the gel was preserved in a water-methanol-acetic acid (5 : 5 : 1) solution.

RESULTS

The results obtained from the present experiments show the remarkable difference in the ADH patterns of the starch gel electrophoresis in the four strains of *Drosophila melanogaster* from Korea.

1. The Chunchun strain: As seen Figure 1, the ADH zymograms of the Chunchun strain shows various numbers of bands (1-5) depending upon the individual flies; five bands appear in two individuals, four in five, three in seven, two in seven, and one in three. The comparison of the zymograms between in males and in females shows that numbers of bands in females (2-5) are greater than in males (1-3), and moreover the bands in females are much thicker in staining intensity than in males, and females exhibit faster mobility than males. The migrations to the cathodal direction are also seen in the most individuals of this strain used and it is noticeable that all of bands appeared in the zymograms are rather smaller in width.

2. The Jinju strain: As illustrated in Figure 2, one to three bands appear in the zymograms; three bands are shown in three individuals, two in six, and one in 15. Numbers of bands in females are greater than in males, mobility in females is faster than in males, and staining intensity shows same degree in both of females and males. No migrations to the cathodal direction are seen in this strain. The bands appeared in the zymograms of this strain are somewhat thicker in width.

3. The Sinchon-Seoul strain: The ADH zymograms show, as seen in Figure 3, one to five bands are presented; five bands in one individual, four in one, one in five, and two in the remaining 17 individuals. The comparison of the electrophoretic patterns between males and females shows the trend opposite to the above two strains: the numbers of band in males (2-5) are greater than in females (1-3), staining intensity is slightly greater in males than in females but mobility is seen same degree in both of males and females. The migration to the cathodal direction is seen in one case in each of males and females.

4. The Taijun strain: The electrophoretic patterns presented in the zymograms of the Taijun strain is found to be somewhat contrast to the above three strains. Variations in the numbers of band, staining intensity, and mobility are not conspicuous compared to the other three strains: all of females show two bands and most of males, three. Staining intensity is uniform in the intermediate degree. But mobility is faster in males than in females and migration to the cathodal direction is seen not in females but in all of males (Fig. 4).

5. The comparison of the four strains: The electrophoretic patterns of the four strains appeared in the present experiment are compared to each other and are presented in Table 1.

Table 1. Comparison of ADH zymograms in the four strains of *Drosophila melanogaster* in Korea.

Strain	No. of bands			Staining intensity	Mobility
	Range	Average No.	Band No. appeared		
Chunchun	1—5 ♀ : 2—5 ♂ : 1—3	2.8 ♀ : 3.7 ♂ : 2.0	B,D,E,F,G,	Variable: thicker, intermediate	Intermediate, migrate also to cathodal direction
Jinju	1—3 ♀ : 1—3 ♂ : 1—2	1.5 ♀ : 1.8 ♂ : 1.3	A,B,E,	Variable: thicker, intermediate, and fainter	Faster
Sinchon-Seoul	1—5 ♀ : 1—4 ♂ : 2—5	2.0 ♀ : 1.7 ♂ : 2.3	B,C,E,F,H	Variable: thicker, intermediate, and fainter	Intermediate, migrate also to cathodal direction
Taijun	2—3 ♀ : 2 ♂ : 2—3	2.4 ♀ : 2.0 ♂ : 2.8	A,B,E,G	Uniformly intermediate	Faster, migrate also to cathodal direction

As illustrated in Table 1, the numbers of bands, staining intensity and mobility are considerably variable in the three strains, Chunchun, Jinju, and Sinchon-Seoul but relatively uniform in the Taijun strain. If the band number is designated such that the fastest migrating anodal band is numbered "A" and numbering is continued in a cathodal direction passing through the origin and ending with the cathodal end of the gel (cf. Brewer 1970), eight band numbers can be made in the zymograms resulted from the present experiment, A,B,C,D,E,F,G, and H. The Chunchun and the Sinchon-Seoul strain show five kinds of band and the Taijun strain, four, and the Jinju strain, three.

Considering the above descriptions, the Taijun strain may be homogeneous and the other three strains, on the contrary, heterogeneous with regard to the ADH constitutions.

DISCUSSION

As mentioned in the results of the present experiment, the electrophoretic patterns of the ADH isozyme are strikingly different each other among the four strains of *D.melanogaster* from Korea. The numbers of bands, staining intensity, and mobility appeared in the zymograms are considerably variable in the Chunchun, the Jinju, and the Sinchon-Seoul strain but relatively uniform in the Taijun strain.

Fig .1

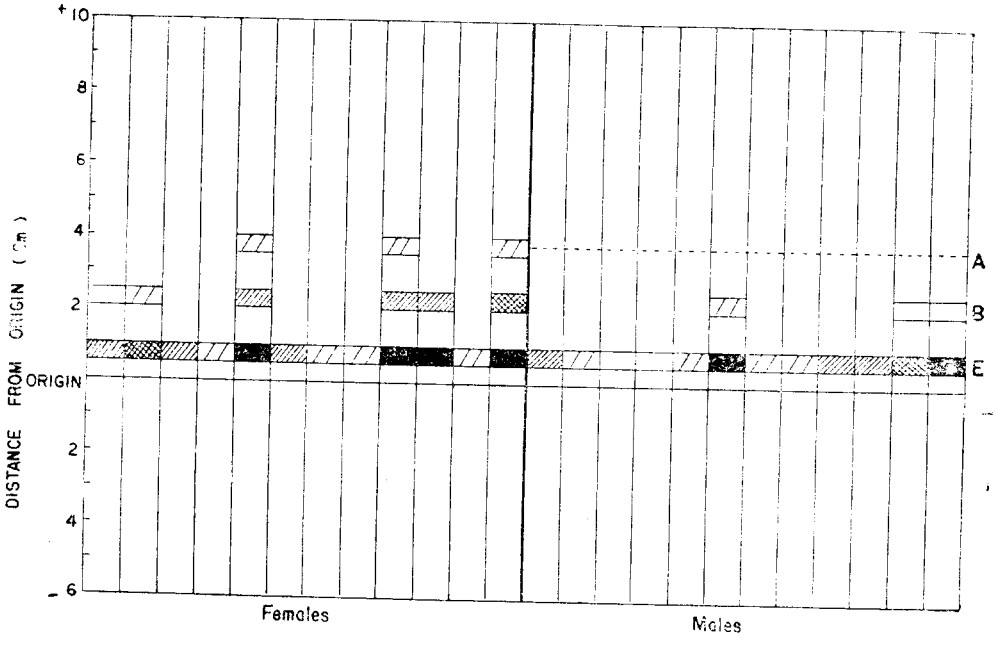

+10
8
6
4
2
ORIGIN
2
4
6
DISTANCE FROM ORIGIN (Cm)
A
B
E
Females
Males

Fig. 2

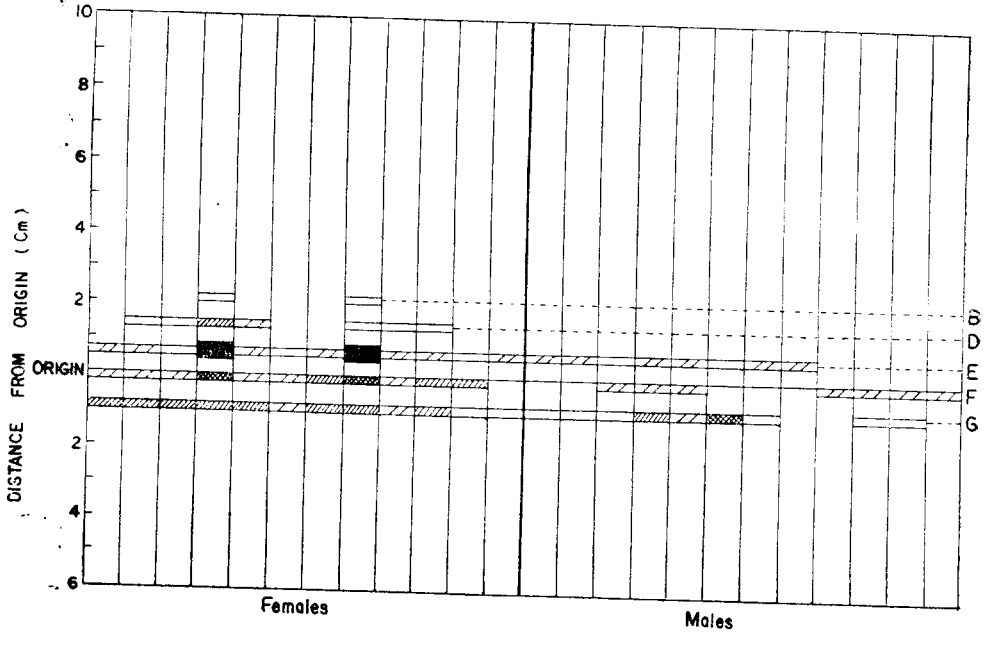

10
8
6
4
2
ORIGIN
2
4
6
DISTANCE FROM ORIGIN (Cm)
B
D
E
F
G
Females
Males

Fig. 3

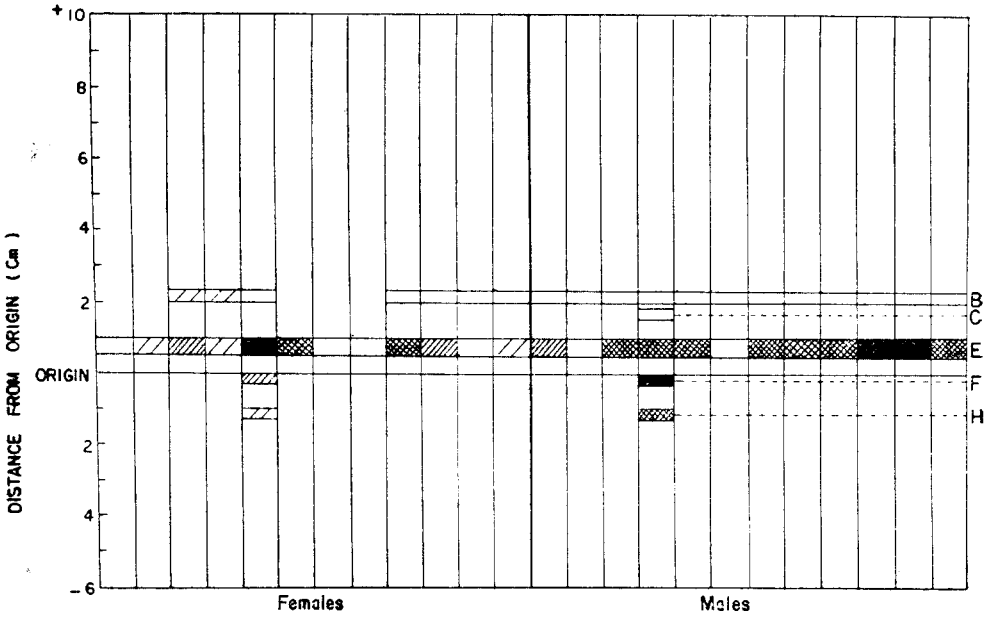
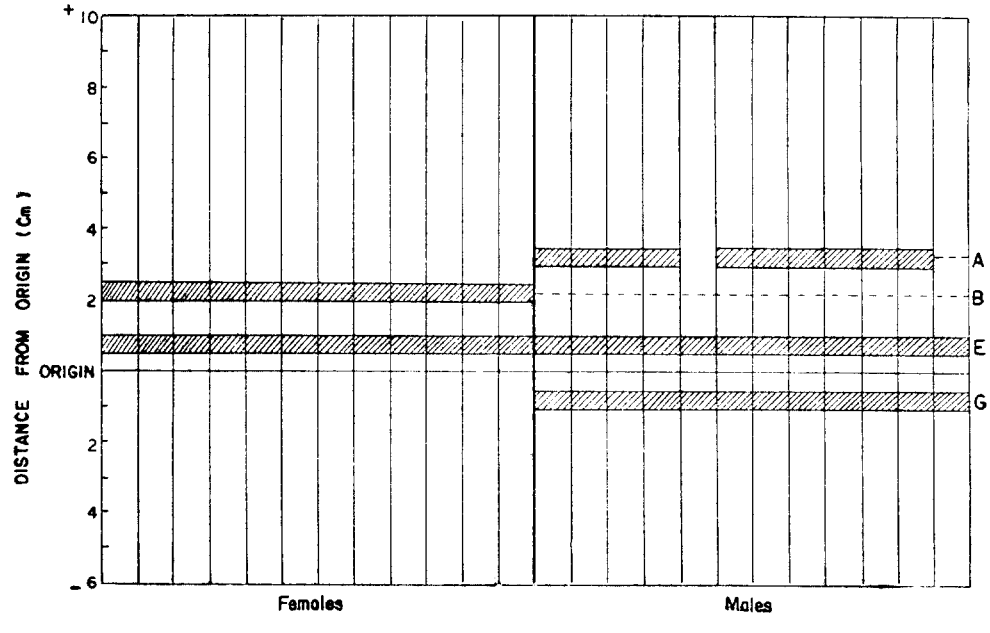

+10
8
6
4
2
ORIGIN
2
4
-6
DISTANCE FROM ORIGIN (Cm)
B
C
E
F
H
Females
Males

Fig. 4


+10
8
6
4
2
ORIGIN
2
4
-6
DISTANCE FROM ORIGIN (Cm)
A
B
E
G
Females
Males

This implies that the Taijun strain is homogeneous but the other three strains are heterogeneous for the ADH constitutions. This difference may be interpreted such that the Taijun strain had been raised in the laboratory for a quite long period of time since captured originally from the natural populations in the Taijun area, resulting in homogeneity for the ADH constitution but the other three strains had elapsed only 5-10 generations between collections and examinations for the alcohol dehydrogenase, still maintaining the heterogeneous conditions.

The present expriment also shows the difference of the ADH zymograms between males and females. This is hard to interpret.

The first investigation of Drosophila alcohol dehydrogenase indicated two (Johnson & Denniston 1964) or three bands (Grell *et al* 1965) in homozygous flies. Ursprung & Leone (1965) reported 3-6 bands and Ursprung & Carlin(1968), 5-7 bands in the zymograms of the ADH isozymes of *D. melanogaster*. The previous work (Chung & Lee 1972) also indicated that three bands were observed in the three strains (Chungju, Jeungpyung, and Taijun), two in the Junju strain, and only one in the remaining 14 strains among total of 18 strains in Korea. The numbers of bands appeared in the present experiment are counted 1-5. Thus it is apparent that the numbers of bands appeared in the zymograms are diverse depending upon the Drosophila ADH investigators (but it is noticeable that the Taijun strain shows 2-3 bands in both of the previous (Chung & Lee, 1972) and of the present experiment).

This disagreement of the numbers of bands, as Chung & Lee (1972) pointed out, may be due to the electrophoretic technique, particularly the kinds of media used. The electrophoretic media used in the present work is starch gel as used in the experiment of Johnson & Denniston (1964) while the previous workers used agar gel (Ursprung & Leone 1965, Ursprung & Carlin 1968), acrylamide gel (Grell *et al* 1965) or cellulose acetate strip (Chung & Lee 1972). Each electrophoretic medium has both of advantage and disadvantage. Since the technique of starch gel electrophoresis was introduced by Smithies (1955), this has been widely used in the study of isozymes than any other method. Powered starch is hydrolyzed by heating in an aqueous solution. As the solution cools it forms a gel. This type of gel contributes an additional factor to the separation of proteins that is absent in other media. The pores in the gel matrix are of the same order of magnitude in size as the protein molecules, resulting in a "molecular sieving" effect which further contributes to protein separation (cf. Brewer 1970). This property leads to greater sensitivity in the resolution of protein bands.

The results of the previous work (Chung & Lee 1972) in which the cellulose acetate strip was used as the electrophoretic media showed only one band in the

most strains used. But the numbers of bands observed in the present experiment carried out with starch gel media are greater (1-5) than in the previous work. This suggests that the starch gel electrophoresis has a resolution power superior to any other type of zone electrophoresis (Poulik 1957).

Many problems remain to be solved. The electrophoretic patterns of the ADH isozyme in the Korean Drosophila strains should be examined by using agar gel or acrylamide gel media and then compare them with the results of starch gel or cellulose acetate electrophoresis. Furthermore the genetic analysis of the ADH isozymes must be done soon on Drosophila strains in Korea.

SUMMARY

The alcohol dehydrogenase (ADH) isozyme of the four strains of *Drosophila melanogaster* in Korea was studied by the starch gel electrophoresis and the results are given below:

1. The numbers of bands, staining intensity and mobility are considerably variable in the three strains, Chunchun, Jinju and Sinchon-Seoul, but relatively uniform in the Taijun strain.
2. It is suggested that the Taijun strain may be homogeneous and the other three strains, on the contrary, heterogeneous for the ADH constitution.
3. The electrophoretic patterns are observed to be different between males and females in all strains used which is hard to interpret.
4. The starch gel electrophoresis is to have a resolving power superior to cellulose acetate electrophoresis.

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