KOREAN JOURNAL OF ZOOLOGY
Vol. 17, No. 3, 117—126 (1974)

A Study of Amylase Isozymes in Five Strains of *Drosophila melanogaster* in Korea

Yong Jai Chung* and Kyung Sook Park**
(*Ewha Womans University, **Osaka University)

한국산 5계통의 초파리(*Drosophila melanogaster*)의 Amylase Isozyme에 관한 연구

鄭 瑢 載* · 朴 京 淑**
(*梨大 師大 科學敎育科, **大阪大學 醫學部 遺傳學敎室)

(Received April 16, 1974)

적 요

한국산 초파리의 생화학적 집단유전학의 체계를 수립하기 위하여 한국의 4개 지역으로 부터 채집, 사육한 초파리(*Drosophila melanogaster*) 5계통, 즉 여수, 충주, 제주, 신촌 III 및 신촌 IV의 amylase isozyme을 polyacrylamide gel 박층 전기영동법으로 분석, 검토한 결과는 아래와 같다.

1. 신촌 IV를 제외한 대부분의 초파리 계통의 전기영동상에 단 하나의 band(Amy^1)가 나타난 경우가 많은데, 이것은 그들 초파리 계통이 amylase에 관하여 homogeneous하다는 것을 의미한다.
2. 신촌 IV 계통은 영동상에 변동이 많은데(대부분 $Amy^{1,2}$와 $Amy^{1,4}$), 이 계통은 amylase에 관하여 heterogeneous 하다는 것을 의미한다.
3. Amylase 계통 중 Amy^1이 가장 보편적으로 분포하고 있다.
4. 잡종실험의 결과는 일정한 결론을 내리기가 매우 곤란하다.
5. 한국의 다른 여러 지역으로 부터도 많은 계통의 초파리를 채집하여 더욱 광범위하게 이 문제를 다룰 계획이다.

INTRODUCTION

Since the discovery of isozymes, the existence of many enzymes in multiple molecular forms within an organism and within a single cell has become a general biological principle. Consequently the investigations of such multiple molecular forms of various enzymes have been extended to many organisms ranging from microorganism to man by many workers. The progress of those isozyme studies is largely due to the improvements in the techniques for protein separa-

tion, such as the method of cellulose acetate-, agar gel-, starch gel-, or acrylamide gel-electrophoresis (Brewer, 1970).

Studies on the isozymes of insects are specially promising because of their remarkably good experimental materials for the investigations of isozymes, particularly from a genetic standpoint of view. For this reason, number of insect geneticists have begun to look for isozymic forms in insects, in particular, house fly (Chung, 1973) and drosophild fly (Chung and Lee, 1972).

The genetic variation in α- amylases has been reported in various drosophilid species by many investigators (Abe, 1958; Kikkawa, 1960 a,b, 1963 a,b,c, 1964, 1965 a,b; Kikkawa and Abe, 1958; Kikkawa and Ogita, 1962; Ogita, 1963; Doane, 1963, 1965, 1966, 1967, 1968 a,b,c,d, 1969 a,b, 1971 a,b; Bahn, 1967, 1971 a,b; McCune 1969 a,b; Pujik, 1972).

Recently the genetic variations in the natural populations of many organisms tend to be analyzed under the protein level which is the primary product of genetic informations as interpretation of hereditary phenomenon by the molecular level and the application of physicochemical methods to the biological science have been progressed. Thus the study of the genetic variations of enzymes is also very significant from the standpoint of population genetics.

Chung and Lee (1972) examined the electrophoretic patterns, mobilities, and activities of the alcohol dehydrogenase (ADH) isozymes in the strains of *Drosophila melanogaster* from 18 localities of Korea with cellulose acetate media and indicated that the isozyme zymograms were different each other among the strains. The ADH was also examined by starch gel electrophoresis (Chung, 1973) and acrylamide gel electrophoresis (Chung and Oh, 1974) and found that the numbers of bands, staining intensity, and mobility were considerably variable among the str-ains of *D. melanogaster* in Korea.

The purpose of the present investigation is to examine the electrophoretic pattern of the amylase isozymes in the strains of *D. melanogaster* from the five localities of Korea by means of acrylamide gel electrophoresis.

MATERIALS AND METHODS

1. *Drosophila stocks:* The five strains of *D. melanogaster* from Korea were used for the present investigation, Yusu, Choongju, Jeju, Sinchon Ⅲ, and Sinchon Ⅳ. The collection sites and dates are illustrated in Table 1.

Flies were captured originally from breweries or traps with fermenting peaches, apples, or grapes. Flies thus obtained had been kept in the constant temperature room (25±1°C) until they supplied the amylase extracts to be used in the present experiments.

Approximately 100 generations of the Yusu strain and about 20 generations of the Sinchon Ⅲ strain had elapsed between collection and the examinations of the amylases. The remaining three strains had been raised for only one or two months since captured originally (particularly the Sinchon Ⅳ strain had been kept for only a few weeks until used in the present experiment), implying that those strains could maintain the features of the natural populations of *D. melanogaster* in those localities of Korea.

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

2. *Electrophoresis:* The electrophoresis was performed in the flat slabs of 5% polyacrylamide thin layer gel (Raymond and Weintraub, 1959).

For the preparations of buffer and gel medium, the following solutions were mixed such that A : B : C=2 : 1 : 1 (vol) and about 20 cc of them was poured into the gel mold (16× 4cm), resulting in a polyacrylamide gel thin layer of 1—1.5 mm.

A: 1 N HCl ..... 5 ml
Tris ..... 4.56 g
Acrylamide ..... 9.5 g
BIS ..... 0.5 g
Deionized water ..... 95 ml

B: TEMED(N,N,N',N'—tetramethylethylene-diamine) ..... 1 ml
Deionized water ..... 100 ml

C: Ammonium persulfate ..... 120 mg
Deionized water ..... 200 ml

And the vessel buffer solution was prepared by mixing the following agents (pH=8.8):

Boric acid ..... 37 g
NaOH ..... 4 g
Deionized water ..... 2,000 ml

In order to prepare the enzyme extracts, a single adult fly was placed and homogenized in a 0.025 ml of phosphate buffer (pH=7.4) in the hole slide glasses. The crude enzymes thus extracted were centrifuged at 0°C for 30 minutes at 12,000 rpm to remove debris and the supernatant immersed strips of filter paper (8×1mm) and placed the gel. The gel was left in 5~10°C for 30 minutes, and then the strips were taken off which was ready for the electrophoresis.

The gel was connected to buffer in electrode vessels with filter paper. An electrophoresis was carried out with 0.8~1.0 mA per cm width of the gel layer

Table 1. The collection records of the five strains of *D. melanogaster* in Korea used in the present investigation.

Strains	Collection sites	Collection dates
Yusu	Inside of breweries located in Yusu City, Chunnam Province	July-September, 1968
Choongju	Inside of breweries located in Choongju City, Choongpuk Province	July-August, 1973
Jeju	Trapping around Samsunghyul located in Jeju City, Jeju Province	June-July, 1973
Sinchon III	Trapping around Sinchon area in Seoul City	July-October, 1972
Sinchon IV	Trapping around Sinchon area in Seoul City	August-November, 1972

for 90 minutes in a low temperature room (5°C).

After electrophoresis the gel was incubated at 37°C in 1% (wt/vol) soluble starch solution containing 0.2% sodium chloride for 90 minutes. After incubation the gel was immersed in 5% (vol/vol) acetic acid for three minutes and stained with I_2-KI solution. This activity was observed as color free band in a purple background and the zymograms were photographed or diagramed.

RESULTS

The results obtained from the present experiments show the unique patterns of the amylase zymograms in the five strains of *D. melanogaster* from Korea.

1. *Comparison of zymograms among the strains*: The electrophoresis was repeated seveal times for each strain and the zymograms were taken pictures or diagramed as seen in Fig. 1—3.

As shown in Fig. 1, zymograms obtained from the Choongju, Jeju, Yusu and Sinchon III strain exhibt uniformly only one band whereas the Sinchon IV strain shows two bands. In order to confirm this difference, the experiments were repeated and the same results were obtained from the four strains (Choongju, Yusu, Jeju, and Sinchon III) showing only one unique band in the zymograms while the Sinchon IV strain presents various band patterns as illustrated in Fig. 2.

If amylase isozyme symbols (Doane, 1969 a. b) are employed to the present zymograms, the isozyme pattern of the four strains presenting only one band is determined to be Amy^1. As Fig. 2 shows, the Sinchon IV strain is involved $Amy^{1,2}$ and $Amy^{1,4}$ in the homozygous and heterozygous states.

When the comparison of zymograms obtained from the present strains to those from the amylase strains which had been kept in the Genetics Laboratory of Osaka University (Fig. 3) was made, the band patterns of present strain show

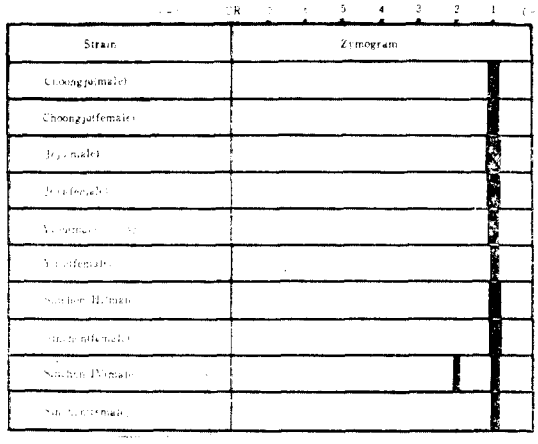

Fig. 1. Diagram showing the zymograms of amylases obtained from five strains of *D. melanogaster* in Korea.

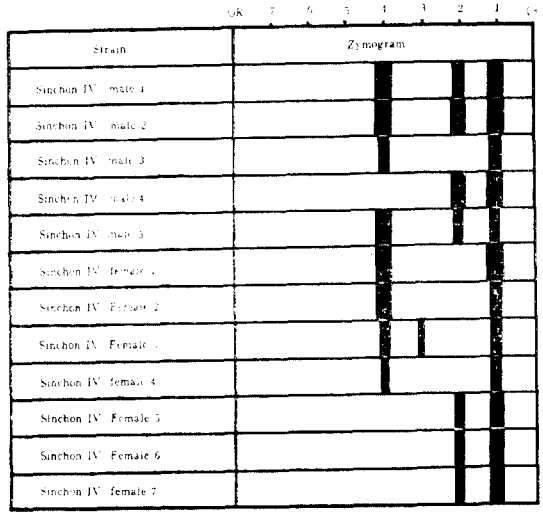

Fig. 2. Diagram showing various patterns of isoamylases in the Sinchon IV strain of *D. melanogaster* in Korea.

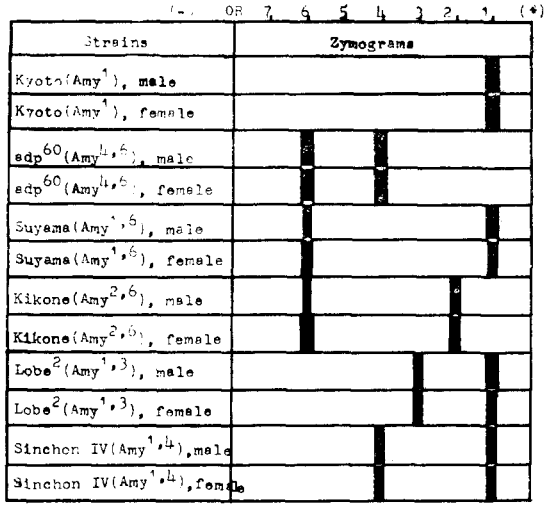

Fig. 3. Diagram showing the comparison of zymograms obtained from the Japanese strains and the Sinchon IV stain of *D. melanogaster* in Korea.

Table 2. The results of hybridization studies.

Cross			F_1 progeny			
Male	X	Female	Band No. appeared	Males	Females	Total
Choongju		Sinchon IV	No. 1	8	14	22
(Amy^1/Amy^1)*		(Amy^1/Amy^1)	No. 1,2	11	9	20
Sinchon IV		Choongju	No. 1	9	15	24
(Amy^1/Amy^1)		(Amy^1/Amy^1)				
Jeju		Sinchon IV	No. 1	2	1	3
(Amy^1/Amy^1)		($Amy^1/Amy^{1,4}$)	No. 1,2	0	1	1
			No. 1,4	1	2	3
Sinchon IV		Jeju	No. 1	4	8	12
($Amy^1/Amy^{1,2}$)		(Amy^1/Amy^1)	No. 1,2	3	4	7
Yusu		Sinchon IV	No. 1	4	4	8
(Amy^1/Amy^1)		($Amy^1/Amy^{1,4}$)	No. 1,4	10	11	21
Sinchon IV		Yusu	No. 1	9	13	22
(Amy^1/Amy^1)		(Amy^1/Amy^1)				
Sinchon IV		Sinchon III	No. 1	18	15	33
($Amy^1/Amy^{1,4}$)		(Amy^1/Amy^1)	No. 1,2	0	1	1
			No. 1,4	15	17	32
Sinchon IV		Sinchon IV	No. 1	2	3	5
(Amy^1/Amy^1)		($Amy^1/Amy^{1,2}$)	No. 1,2	4	3	7

* Descriptions in parenthesis indicate the possible genotypes for the amylase isozymes.

the remarkable contrast to those in Japan.

Judging from the above descriptions, the Yusu, Choongju, Jeju and Sinchon III strain may be homogeneous whereas the Sinchon IV strain heterogeneous for the amylase constitutions.

2. *Hybridization studies*: When any two different strains were mated to each other, the F_1 individuals showed the mixtures of the parental patterns with a few exceptions (Table 2). However the distribution of the band patterns in F_1 progeny, as seen in Table 2, is so complicated that any definite conclusion could not be established. Nevertheless it may be confirmed once again that the Sinchon IV strain is heterogeneous for the the amylase isozyme constitutions.

DISCUSSION

Recently many investigations concerning the existence of isozymes of various enzymes in many species of organisms have been reported. Among them, the amylase isozymes found in drosophilid species are particularly complicated and unique.

Thus far, the amylases of 42 species of Drosophila covering 18 species groups have been analyzed electrophoretically (nine by Kikkawa, 1963 b, 1964, 1965 a; the remainder by Doane, 1968 d).

The progress in the isozyme studies is largely due to the improvements in the electrophoretic techniques. For instance, various kinds of electrophoretic media were developed for the amylase study on the drosophilid flies: agar gel (Kikkawa and Ogita, 1962; Kikkawa, 1963 a,b,c, 1964, 1965 a,b; Ogita, 1963 ; Bahn, 1967), acrylamide gel (Doane, 1965, 1966, 1967, 1968 c, 1969 a), and recently McCune (1969 a, b) devised a 7% cyanogum gel electrophoresis. Among them thin layer polyacrylamide gel has been recognized as the superior electrophoretic medium (Ogita, 1965, Ogita *et al*, 1966, Doane, 1967) which was employed in the present experiment.

Genetic control of amylase in *D. melanogaster* was first described by Kikkawa and his coworkers (Abe, 1958; Kikkawa, 1960 a,b; Kikkawa and Abe, 1958) who suggested a single controlling locus on the right arm of the second chromosome. The most recent estimates place the *Amy* region at 2-77.3 (Doane, 1963, 1969 a), 2—78.1 (Kikkawa, 1964) and 2—77.9 (Bahn, 1967).

Since Kikkawa (1964) separated seven kinds of isoamylases, a multiple forms of amylase in *D. melanogaster* (symbols: Amy^1, $Amy^{1,3}$, Amy^4, $Amy^{1,6}$, $Amy^{2,6}$, $Amy^{3,6}$, $Amy^{4,6}$), $Amy^{1,2}$ (Doane, 1967), $Amy^{2,3}$ (Bahn, 1967) and Amy^5 (Pujik and de Jong, 1972) were added, resulting in a total of ten kinds of isoamylases.

The present strains except the Sinchon IV one exhibit only one band (Amy^1)

in the zymograms. Kikkawa (1964) indicated that the amylase pattern, *Amy*^{1} was predominated and McCune (1969 a) also reported that the *Amy*^{1} gene was the most common in natural populations of *D. melanogaster*. Thus it is highly conclusive that the *Amy*^{1} is the commonest one among isoamylase loci.

As Kikkawa (1964) described, studies on amylase isozymes in *D. melanogaster* may contribute the population genetics of this species, as lethal gene or inversions did in this field. Chung and Lee (1972) and Chung (1973) examined the alcohol dehydrogenase (ADH) variations in *D. melanogaster* strains of various localities in Korea from the population genetic point of view. The present work also is an attempt to find a population genetic system as to amylase isozyme in this species.

The interpretation of the trend that various numbers of bands appeared in the zymograms of the Sinchon IV strain may be made such that only a few weeks had elapsed between the collection of flies and the amylase studies to maintain the original features of natural populations in Sinchon area, resulting in heterogeneous conditions of amylase constitutions.

According to Kikkawa (1964) and Doane (1969 b), hybrids from cross between any two *Amy* strains produced banding patterns that were a composite of the parental types, with no hybrid enzymes formed. The present experiment also was designed to examine this trend but it was hard to establish a definite conclusion since the results were not simple.

The amylase study on the Korean strains of *D. melanogaster* should be extended to many other strains from the various localities other than the present sites of collections and the detailed analysis of *Amy* strains also should be done in future.

SUMMARY

The amylase isozyme of the five strains of *D. melanogaster* in Korea (Yusu, Choongju, Jeju, Sinchon III and Sinchon IV) was examined by the polyacrylamide thin layer gel electrophoresis and the results obtained are presented below:

1. The most strains except the Sinchon IV show only one band (*Amy*^{1}) in the zymograms. This implies that those strains may be homogeneous for amylase constitutions.

2. The Sinchon IV strain exhibits variable band patterns (mostly *Amy*^{1,2} and *Amy*^{1,4}) suggesting that this strain may be of heterogeneous amylase constitutions.

3. The *Amy*^{1} strain may be the commonest one in the Korean *D. melanogaster*

populations as in other countries.

4. The results of hybridization studies are hard to interpret.

5. Further studies will be extended to many other strains from various localities of Korea.

REFERENCES

Abe, K., 1958. Genetical and biochemical studies on amylase in *D. melanogaster*. *Jap. J. Genet.* **33** : 138—145 (in Japanese).

Bahn, E., 1967. Crossing over in the chromosomal region determining amylases in *Drosophila melanogaster*. *Hereditas* **58** : 1—12.

——, 1971 a. Cytogenetical localization of the amylase region in *Drosophila melanogaster* by means of translocations. *Hereditas* **67** : 75—78.

——, 1971 b. Position-effect variegation for an isoamylase in *Drosophila melanogaster*. *Hereditas* **67** : 79—82.

Brewer, G.J., 1970. Introduction to isozyme techniques. Academic Press, New York.

Chung, Y.J., 1973. A study of alcohol dehydrogenase of *D. melanogaster* in Korea by starch gel electrophoresis. *Kor. J. Zool.* **16** : 67—77.

——, and K.S. Lee, 1972. A biochemical and genetic study of alcohol dehydrogenase in the Korean natural populations of *Drosophila melanogaster*. *J. Kor. Res. Inst. Better Living* **9** : 123—132.

——, and S.H. Oh, 1974. A study of alcohol dehydrogenase of *Drosophila melanogaster* in Korea by means of acrylamide gel electrophoresis. *J. Kor. Res. Inst. Better Living* **13** (in press).

Doane, W.W., 1963. Linkage data for *Amy* and *adp* mutant of *D. melanogaster*. *DIS* **38** : 32.

——, 1965. Use of disc electrophoresis for analysis of amylase isozymes. *DIS* **40** : 97.

——, 1966. Disc electrophoresis of α-amylases in individual Drosophila. *DIS* **41** : 93—94.

——, 1967. A novel approach to the analysis of enzymes separated by disc electrophoresis. *SABCO Journal* **3** : 63—68.

——, 1968 a. Amylase (*Amy*): symbols, cytogenetics. *DIS* **43** : 66.

——, 1968 b. Observations relating duplications in the amylase region of *D. melanogaster*. *DIS* **43** : 105.

——, 1968 c. The amylase locus in *Drosophila hydei*. *DIS* **43** : 143.

——, 1968, d. Isozymes as tools for the geneticst. *Proc. III Int. Congr. Histochem. Cytochem. New York,* 1968 : 37—39.

——, 1969 a. Amylase variants in *Drosophila melanogaster*: Linkage studies and characterization of enzyme extracts. *J. Exp. Zool.* **171** : 321—342.

——, 1969, b. Drosophila amylases and problems in cellular differentiation: In RNA in development, E.W. Hanly, ed. *Univ. of Utah Press, Salt Lake City*: 73—103.

——, 1971 a. Isoamylase in *Drosophila hydei*: a system for the analysis of gene-specific

puffing activity. *DIS* **47** : 100.

——, 1971 b. X-ray induced deficiencies of the amylase locus in *Drosophila hydei*. *Isozyme Bull.* **4** : 46—47.

Kikkawa, H., 1960 a, Genetic control of amylase in *D. melanogaster*. *DIS* **34** : 89.

——, 1960 b. Further studies on the genetic control of amylase in *D. melanogaster*. *Jap. J. Genet.* **35** : 382—387.

——, 1963, a. Agar-gel electrophoretic studies on amylase in *D. melanogaster*. *DIS* **37** : 94.

——, 1963 b. The genetic study on amylase in *Drosophila virilis*. *Ann. Rep. Sci. Works, Fac. Sci. Osaka Univ.* **11** : 41—50.

——, 1963 c. An agar-gel electrophoretic study on amylase in *D. virilis*. *DIS* **38** : 88.

——, 1964. An electrophoretic study on amylase in *D. melanogaster*. *Jap. J. Genet.* **39** : 401—411.

——, 1965, a. An electrophoretic study on amylase in *Drosophila simulans*. *SABCO Journal* **1** : 37—41.

——, 1965 b. An electrophoretic analysis of amylase in *D. simulans*. *DIS* **40** : 72.

——, and K. Abe, 1958. Genetic control of amylase in *D. melanogaster*. *DIS* **32** : 131.

——, and Z. Ogita, 1962. Biochemical and genetical analysis of amylase in Drosophila by means of agar-gel electrophoresis. *Jap. J. Genet.* **37** : 394—395.

McCune, T., 1969 a. Amylase isozymes in natural populations of *Drosophila melanogaster*. *DIS* **44** : 77—78.

— , 1969 b. Electrophoresis of amylases of *Drosophila melanogaster*. *DIS* **44** : 125.

Ogita, Z., 1963. Enzyme separation by agar-gel electrophoresis in *D. melanogaster*. *DIS* **37** : 142.

——, 1965. Disc-electrophoresis (discontinuous electrophoresis). *Metabolism and Disease* **2** : 63—79 (in Japanese).

——, M. Hashinotsume, and Y. Kosugi, 1966. The polyacrylamide gel electrophoresis. *SABCO Journal* **2** : 58—65 (in Japanese).

Pujik, K., and G. de Jong, 1972. α-amylase in a population of *D. melanogaster* from Dahomey. *DIS* **49** : 61.

Raymond, S., and L. Weintraub, 1959. Acrylamide gel as supporting medium for zone electrophoresis. *Science* **130** : 711.