Korean J. Zool. 37:249-254, 1994

Genetic relationships and protein variations during development within the *Drosophila melanogaster* species group. II. Analysis of soluble protein by 2DE

Taek Jun Lee, Kyoung Ja Hong and Nam Woo Kim

Department of Biology, Chung-Ang University, Seoul, 156-756, Korea

Soluble protein of the eight species of the *Drosophila melanogaster* species group was analyzed for three developmental stages of larva, pupa and adult by 2DE. Genetic distances were calculated by Aquadro and Avise's equation for three developmental stages, respectively. The dendrograms showed the same patterns in three stages. The dendrograms showed that the *melanogaster* species group consisted of two clustered groups. Total soluble protein contents on three developmental stages of the eight species were compared. Closely related species showed resemblant protein changing pattern during development, and their developmental changing patterns were different according to the subgroup.

KEY WORDS: Genetic relationship, *D. melanogaster*, development, soluble protein.

D. melanogaster species group is one member of the subgenus *Sophophora* which is grouped into 7 species groups. In this species group, 150 species were described and subdivided into 11 subgroups. 12 species of this species group were described in Korea (Lee, 1993) and they occupy 9.3% of 115 species in Korean *Drosophila*. O'Farrell (1975) reported that 2DE (two-dimensional electrophoresis) was an useful technique for detecting proteins in organisms. Aquadro and Avise (1981) reported that it could be an useful adjunctive tool in systematics using rodent speices. They also suggested an approach for quantifying the genetic similarity among different populations from data obtained by 2DE. Ohnishi *et al.* (1983) reported genetic relationships among 29 species in the *Drosophila montium* subgroup with 2DE. Lee and Pak (1985), and Lee and Joo (1987) reported biochemical relationships among several species, habitating in Korea, of *D. auraria* complex and *quinaria* group, respectively. Kim (1988) investigated the phylogenetic relationships of the seven species of the *D. auraria* complex based on the genetic distances by 2DE. Phylogenic relationships among the eight species of the *Drosophila melanogaster* group from Korean natural populations were analyzed by Kim and Lee (1991), Kim *et al.* (1992), and Kim *et al.* (1992) using various methods of allozyme analysis, taximetric analysis and protein analysis. In a way, studies on the developmental patterns of enzyme activities and protein variations for some species have been performed (McDonald and Avise, 1975; Pasteur and Kastritsis, 1971; Lee and Ahn, 1990). However, researches which were considered together both aspects of phyletic relationships and intraspecific developmental changing pattern, have been seldom accomplished. In this study, phylogenetic relationships of the eight species of *melanogaster*

This is part of the work supported by a grant (No. 921-0400-038-1, 1992) from the Korean Science and Engineering Foundation.

species group were analyzed on three developmental stages and qualitative differences during development were compared from the results by 2DE. Additionally, the total soluble protein contents on three developmental stages were estimated by the method of Lowry *et al.* (1951) and the results for the eight species of *D. melanogaster* species group were compared with one another.

Materials and methods

The list of the eight species used in this experiment and their collecting localities are as follows;

1. *D. melanogaster*	Kimpo
2. *D. simulans*	Kimhae
3. *D. lutescens*	Mt. Sori
4. *D. suzukii*	Mt. Chiak
5. *D. auraria*	Kimpo
6. *D. biauraria*	Mt. Sori
7. *D. triauraria*	Mt. Sori
8. *D. rufa*	Wando.

The flies were maintained as the isofemale lines on cornmeal molassess standard medium at the temperature of 25±1°C. The two-dimensional electrophoresis (2DE) was performed as outlined by O'Farrell (1975) with some modification. Ten of 3rd larvae, eight late pupae and seven adult males (4 days old) were sampled from each species. After the samples were homogenized and centrifuged (10,000 rpm, 30 min.), 100 ml of the supernatant was loaded onto an isoelectric focusing gel. The isoelectric focusing was accomplished for a total of 7600v · hrs. (200v-2hrs. 400v-16hrs. 800v-1hr.) after prerunning. SDS slab gel electrophoresis in the second dimension was carried out in 8% acrylamide for 6 hours at 25 mA per one slab gel. After running, all slab gels were stained in the solution containing 0.02% coomassie brilliant blue R-250. They were destained in a mixture of 25% methanol and 7% acetic acid. Genetic distance was calculated according to Aquadro and Avise's equation (1981); $D = 1 - F = 1-2n_x/(n_x + n_y)$, where n_{xy} is the number of shared spots in both species, and n_x or n_y is the total number of scored loci in each species. From the genetic distance data was obtained by 2DE, a phylogenetic tree was constructed according to the unweighted pair group method (UPGMA) of clustering by Sokal and Sneath (1963). 5 flies for each life stage were carefully weighed and homogenized in 400 ml of 0.05M Tris-HCl buffer (pH 8.5). After centrifugation (5000 rpm, 15 min.), 20 ml of supernatant was used to quantify soluble protein contents. The protein contents of each sample were estimated by the method of Lowry *et al.* (1951), using bovine serum albumin (Sigma) as a standard protein.

Result

Genetic distances (D=1-F) between species for three developmental stages obtained by 2DE are shown in Tables 1, 2, and 3. At the 3rd larval stage, genetic distances lie in a range of 0.117-0.557. The smallest value of D (0.117) is found between *D. auraria* and *D. triauraria* followed by

Table 1. Genetic distance in the larval stage among the eight species of the *D. melanogaster* species group obtained by 2DE.

	sim	*lut*	*suz*	*aur*	*bia*	*tri*	*ruf*
mel	0.1969	0.3659	0.5333	0.5214	0.5345	0.5000	0.5372
sim		0.3906	0.5217	0.5574	0.5537	0.5372	0.5397
lut			0.4328	0.4407	0.4701	0.4701	0.5082
suz				0.4531	0.5246	0.5118	0.5152
aur					0.1532	0.1171	0.3276
bia						0.1636	0.3043
tri							0.3043

D=0.153 for *D. auraria* and *D. biauraria*. The most distant relationship was found between *D. simulans* and *D. auraria* (D=0.557). In the results for pupal stage, relationships between *D. auraria* and *D. triauraria* (D=0.154), *D. biauraria* (D=0.190) are the crossest ones. In the case of adult stage, the smallest value of genetic distance was that between *D. auraria* and *D. triauraria* (D=0.144) and farthest value was found between *D. simulans* and *D. biauraria*. From the matrix of genetic distances by 2DE, dendrograms was constructed (Fig. 1) following the UPGMA method (Sokal and Sneath,1963). All dendrograms for three developmental stages showed the same patterns, although their genetic distances were different. First, *D. auraria* and *D. triauraria* were

Table 2. Genetic distance in the pupal stage among the eight species of the *D. melanogaster* species group obtained by 2DE.

	sim	*lut*	*suz*	*aur*	*bia*	*tri*	*ruf*
mel	0.2013	0.3187	0.4433	0.4186	0.4023	0.4884	0.4725
sim		0.3111	0.4583	0.4824	0.4419	0.4824	0.4222
lut			0.3830	0.3976	0.3571	0.3735	0.4773
suz				0.4157	0.4000	0.4831	0.4631
aur					0.1899	0.1538	0.3253
bia						0.2152	0.3095
tri							0.3012

Table 3. Genetic distance in the adults stage among the eight species of the *D. melanogaster* species group obtained by 2DE.

	sim	*lut*	*suz*	*aur*	*bia*	*tri*	*ruf*
mel	0.2113	0.3364	0.4389	0.4300	0.4300	0.4476	0.4378
sim		0.3458	0.4220	0.4418	0.4502	0.4300	0.4299
lut			0.3874	0.4231	0.4326	0.4028	0.4220
suz				0.4434	0.4247	0.4326	0.4144
aur					0.1707	0.1443	0.3173
bia						0.1923	0.6602
tri							0.3175

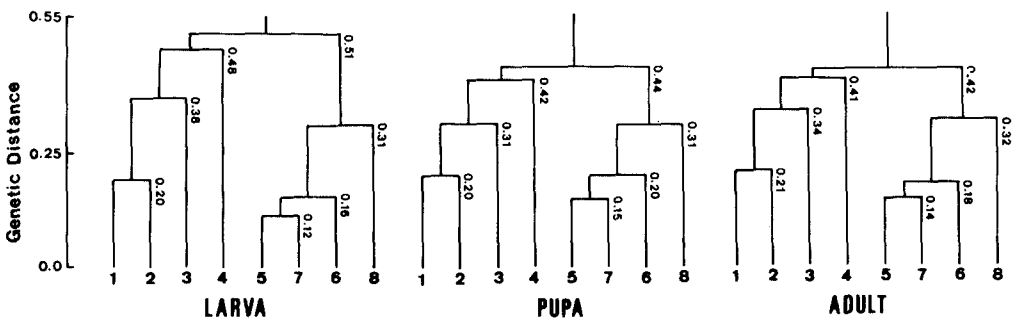

Fig. 1. A dendrogram showing the phylogenic relationship among the eight species of the *D. melanogaster* species group at three developmental stages, based on data of genetic distance obtained by 2DE. 1. *D. melanogaster* 2. *D. simulans* 3. *D. lutescens* 4. *D. suzukii* 5. *D. auraria* 6. *D. biauraria* 7. *D. triauraria* 8. *D. rufa*

clustered and after then *D. biauraria* and *D. rufa* were clustered to former in order. At the other side, *D. melanogaster* and *D. simulans* are first clustered and *D. lutescens* and *D. suzukii* were clustered in succession to form the other group. Table 4 presents the quantities of soluble protein per unit weight for the three developmental stages of eight species. In every eight species, adults contained the smallest protein contents. The highest value was obtained in the larval stage of *D. suzukii* (51.575) and the lowest was appeared in the adult stage of *D. auraria*. Larvae (51.575) and pupae (47.023) of *D. suzukii* were confirmed to have even more protein contents than any other species. Developmental protein changing patterns were devided into two groups. *D. auraria, D. biauraria* and *D. triauraria* which are morphologically related species, and *D. lutescens* and *D. suzukii* showed same tendency that larvae have the most protein contents to be followed by larvae and adults. On the other hand, *D. melanogaster* and its sibling species, *D. simulans* showed the same patterns of protein contents increased in order of pupae, larvae and adults.

Discussion

After the electrophoresis method had been introduced in the population genetics in 1960, the genetic variation of the individuals in the natural population can be investigated at the molecular level. Especially, a great deal of research has been concerned with the genetic variation of *Drosophila* (Singh *et al.*, 1982; Singh and Rhomberg, 1987 a, b; Spicer and Fleming, 1991). Ohnishi *et al.* (1983) had pointed out that a dendrogram based on the data obtained by 2DE apeared to be the most reliable for systematic considerations and is supported by data from interspecific hybridization and allozymes. Ohnishi and Watanabe (1984) investigated genetic relationships among 29 species in the *Drosophila montium* species subgroup using 2DE and constructed a dendrogram of them. Lemeunier *et al.* (1986) summarized the general features of eight species belonging to the *D. melanogaster* subgroup. They gave precise descriptions on taxonomy, chromosomes hybridization and ecology. In this study, soluble protein analysis of the eight species of *D. melanogaster* species group were performed by 2DE. Three dendrograms of eight species of *D. melanogaster* species group were constructed according to their developmental stages based on the above data. At the larval and pupal stages, about 70 protein spots were scored and at the adults stage, about 100 protein spots were scored as present or absent among the eight species. In every cases, dendrograms showed the same pattern although each degree of genetic distance was different according to the developmental stage. The dendrograms might suggest that they were coincident with morphological studies and the *melanogaster* species group consist of two clustered groups; the 1st group of *D. melanogaster, D. simulans, D. lutescens* and *D. suzukii* and 2nd group of *D. auraria, D. biauraria, D. triauraria*, and *D. rufa*. This accordance of dendrograms obtained from three

Table 4. Soluble protein quantities(mg/ml/weight) on three developmental stages of the eight species within *D. melanogaster* species group.

	larvae	pupae	adults
mel	32.879 ± 2.098	43.618 ± 2.029	27.715 ± 0.248
sim	29.826 ± 3.360	35.062 ± 5.012	27.637 ± 4.812
lut	33.546 ± 2.031	28.165 ± 1.212	24.479 ± 1.248
suz	51.575 ± 4.782	47.023 ± 3.510	29.700 ± 2.669
aur	35.383 ± 1.295	33.125 ± 5.505	20.444 ± 2.995
bia	43.215 ± 6.331	29.124 ± 1.881	26.139 ± 4.179
tri	37.798 ± 2.631	28.287 ± 1.605	24.642 ± 3.125
ruf	31.471 ± 3.459	29.779 ± 2.353	27.455 ± 1.858

developmental stages pointed out that protein analysis by 2DE is useful tool to analyze interspecific relationships applicable to any developmental stages of other insects, in spite of their developmental variations. In analysis of quantification of soluble protein contents, their developmental changing patterns were different among the subgroup. They could be divided into two groups of developmental protein chainging patterns. Closely related species showed more resemblant patterns. *D. melanogaster* and *D. simulans* which are sibling species, showed similar protein changing pattern that pupae have the most protein contents to be followed by larvae and adults. *D. auraria, D. biauraria,* and *D. triauraria* of *D. auraria* complex and *D. lutescens* and *D. suzukii* showed same developmental changing patterns increased in the order of larvae, pupae, and adults. It was noticeable that the species belonging to same subgroup showed same changing pattern of soluble protein contents during development.

References

Aquadro, C.F. and J.C. Avise, 1981. Genetic divergence between rodent species assessed by two-dimensional electrophoresis. *Proc.Acad. Sci. USA* **87:** 3784-3788.

Kim, B.K., 1988. Phylogenetic relationship of the seven species of the *Drosophila auraria* complex by two-dimensional electrophoresis. *Korean J. Genetics* **10:** 77-84.

Kim, N.W. and T.J. Lee, 1991. Genetic relationships among the eight species of *Drosophila melanogaster* species group by allozyme analysis. *Korean J. Genetics* **13:** 297-309.

Kim, N.W., T.J. Lee and E.S. Song, 1992. Evolutionary genetic study on the eight species of the *Drosophila melanogaster* group from Korea: Reproductive isolation and protein analysis. *Korean J. Zool.* **35:** 211-218.

Kim, N.W., T.J. Lee and K.J. Hong, 1992. A systematic study on the eight species of the *Drosophila melanogaster* species group by taximetric analysis. *Korean J. Entomol.* **22:** 13-22.

Lee, T.J., 1993. Evolution in *Drosophila*. The Chung-Ang University press.

Lee, T.J. and G.J. Joo, 1987. Genetic relationships within the *quinaria* species group of the genus *Drosophila*. *Korean J. Genetics* **9:**11-18.

Lee, T.J. and J.B. Ahn, 1990. Comparisons of esterase and soluble protein zymogram with developing in *Drosophila auraria* complex. *Korean J. Entomol.* **20:** 131-137.

Lee, T.J. and J.H. Pak, 1985. Biochemical phylogeny of the *Drosophila auraria* complex. *Korean J. Genetics* **7:** 184-192.

Lemeunier, F., J.R. David, L. Tasacas and M. Ashburner, 1986. The *melanogaster* species group. The genetics and biology of *Drosophila*. (M. Ashburner, H.L. Carson and J.N. Thompson, Jr., eds.), Vol. 3*e*. pp. 147-256.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.

McDonald, F.J. and C.J. Avise, 1975. Evidence for the adaptive significance of enzyme activity levels: interspecific variation in α-GPDH and ADH in *Drosophila*. *Bioch. Genet.* **14:** 347-355.

O'Farrell, P.H., 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250:** 4007-4021.

Ohnishi, S., K.W. Kim and T.K. Watanabe, 1983. Biochemical phylogeny of the *Drosophila montium* species subgroup. *Jpn. J. Genet.* **58:** 141-151.

Ohnishi, S. and T.K. Watanabe, 1984. Systematics of *Drosophila montium* species subgroup; a biochemical approach. *Zool. Sci.* **1:** 801-807.

Pasteur, N. and C.D. Kastritsis, 1971. Developmental studies in *Drosophila*. I. Acid phosphatases, esterases, and other proteins in organs and whole-fly homogenates during development of *D. pseudoobscura*. *Devel. Bio.* **26:** 525-536.

Singh, R.S., D.A. Hickey and J. David, 1982. Genetic differentiation between geographically distant populations of *Drosophila melanogaster*. *Genetics* **101:** 235.

Singh, R.S. and L.R. Rhomberg, 1987a. A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. I. Estimates of gene flow from rare alleles. *Genetics* **115:** 313.

Singh, F.S. and L.R. Rhomberg, 1987b. A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic differentiation. *Genetics* **117:** 255.

Sokal, R.E. and P.H.A. Sneath, 1963. Principles of numerical taxonomy. Freeman, San Francisco.

Spicer, G.S and J.E. Fleming, 1991. Genetic differentiation of *Drosophila melanogaster*

populations as assessed by two-dimensional electrophoresis. *Biochem. Genet.* **29:** 389-401. (Accepted February 28, 1994)

노랑초파리종군의 발생단계에 따른 단백질의 변화와 유전적 유연관계
2. 2DE에 의한 수용성 단백질의 분석

이택준 · 홍경자 · 김남우(중앙대학교 이과대학 생물학과)

노랑초파리종군 8종의 수용성 단백질을 2DE법으로 유충, 번데기, 성체의 발생단계별로 분석하였다. 전기영동실험 후 Aquadro 와 Avise의 공식에 따라 세 발생단계별로 8종간의 유전적 거리를 산출하고 dendrogram을 작성하였다. 세 발생단계에서 종간의 유전적 거리에는 약간의 차이를 나타냈으나 모두 같은 pattern의 dendrogram이 작성되었다. 세 발생단계에서 모두 같은 pattern의 dendrogram이 얻어진 것으로 보아 2DE에 의한 단백질 분석에 의한 종간유연관계의 분석은 각 발생단계별로 적용할 수 있는 유용한 방법이 될 수 있을 것으로 생각된다. 발생단계에 따른 수용성 단백질의 양적 변화 pattern을 비교한 결과 종군간의 차이를 관찰할 수 있었다.