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Phylogeny of Subgenus *Drosophila* (Drosophilidae: *Drosophila*) in Korea by Allozyme and Soluble Protein Analysis

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ABSTRACT

This study was conducted to ascertain interspecific relationships by analyzing allozyme and soluble proteins of ten species in the *Drosophila* (*Drosophila*) to form a part of systematic studies of Korean drosophilids. The results of allozyme and TDE analysis showed that *D.* (*D.*) *curvispina* and *D.* (*D.*) *tsigana* had the furthest genetic distance. On the other hand, the genetic distance between *D.* (*D.*) *angularis* and *D.* (*D.*) *brachynephros* was extremely close. And, ten species of the subgenus *Drosophila* can be divided into the first group of *D.* (*D.*) *virilis*, *D.* (*D.*) *tsigana* and *D.* (*D.*) *lacertosa*, and the second group consisted of four subgroups; the first subgroup clustered *D.* (*D.*) *angularis* and *D.* (*D.*) *brachynephros*, the second subgroup clustered *D.* (*D.*) *unispina* and *D.* (*D.*) *curvispina*, the third subgroup of *D.* (*D.*) *takadai* and *D.* (*D.*) *kuntzei* and the fourth subgroup of *D.* (*D.*) *nigromaculata* alone.

Key words: *Drosophila*, allozyme, soluble protein, Korea

INTRODUCTION

Drosophilidae are divided in multi-levels distributed worldwide. Any study on the relationship considering their ecogeological characters can be pivotal in speciation studies. For phylogenetic study on Drosophilidae, morphological characters, ecological, reproductive isolation, and molecular

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biological studies have been carried out.

Genus *Drosophila* includes a vast number of species and various specialized species that are distributed in different regions. Subgenus *Drosophila* of Genus *Drosophila* includes 22 species groups and there are approximately 800 known species in the world (Wheeler, 1986). There are 23 species of 12 species groups in Korea and they are about 20% of 117 species of Korean Drosophilidae (Lee and Kim, 1987; Kim and Joo, 2002).

Allozyme was used as one of the methods in ascertaining the interspecific relationships. For studies on Drosophilidae using allozyme, there were studies on *bipectinata* subgroup (Yang et al., 1972), *montium* subgroup (Ohnish et al., 1982), *melanogaster* subgroup (Kim and Lee, 1991), and others. In addition, the analysis of soluble proteins was widely also accepted in the study of interspecific relationships.

Brown and Langly (1979) presented a new evaluation process for heterozygosity of *D. melanogaster* using two-dimensional electrophoresis (TDE). Aquadro and Avise (1981) computed the genetic distance using TDE and compared the genetic distance obtained from allozyme analysis on eight species of rodent and Ohnish et al. (1983) on *montium* subgroup respectively reporting that TDE was an useful adjunctive tool in the systematic study. In Korea, Lee and Pak (1985) and Kim (1988) on *D. auraria* complex, and Kim et al. (1992) on *D. melanogaster* species group released results on the biochemical relationships through TDE. Lee and Joo (1987) imparted on a subgenus *Drosophila*, five *quinaria* species group based on the results of SDS-PAGE and TDE analysis, *D. angularis* and *D. brachynephros* were most closely related. They affirmed that *D. nigromaculata* was the furthest in relationship from other four groups. As for the reports on evolutionary relationship using allozyme and TDE analysis, Kim and Hong (1997) published on the genus *Scaptomyza* and the study on six species of subgenus *Drosophila* by Kim and Song (1999). However, there were no studies on the 10 species of subgenus *Drosophila* used.

This study was conducted to ascertain interspecific genetic relationships by analyzing allozyme and soluble proteins of ten species including three *virilis* section species (*D. virilis, D. tsigana*, and *D. lacertosa*) and seven *quinaria* section species (*D. angularis, D. brachynephros, D. curvispina, D. kuntzei, D. nigromaculata, D. takadai* and *D. unispina*) in the subgenus *Drosophila* to form a part of systematic studies of Korean Drosophilidae.

MATERIALS AND METHODS

Ten species of *Drosophila* used in this study were collected in Inchon (*D. virilis*), Mt. Chonma (*D. tsigana*), Mt. Sori (*D. lacertosa, D. angularis, D. brachynephros* and *D. unispina*), Mt. Jiri (*D. curvispina*), Mt. Chiak (*D. kuntzei* and *D. nigromaculata*) and Daegwallyeong (*D. takadai*) of Korea, respectively.

The allozyme analysis using starch gel electrophoresis was performed as outlined by Shaw and Prasad (1970) with some modification. *Got* and *Mdh* performed the electrophoresis for 3.5 hours at 150 V in tris citrate II buffer. *Acph* and *Adh* performed the electrophoresis for 3.5 hours at 220 V in poulik buffer. Esterase performed the electrophoresis for 1.5 hours at 250 V in phosphate buffer. Interspecific relationship by the allozyme pattern was examined through computation of

Rogers' (1972) genetic similarity. TDE were performed as prescribed by O'Farrell's method (1975) and samples were 8 males from each species. Isoelectroforcusing gel with ampholine pH 3.5-10 and pH 5-8 were used. For total 7,600 Vhrs (200 V-2 hrs, 400 V-16 hrs, 800 V-1 hr), 1st electrophoresis was performed. TDE were performed for approximately 6 hours per slab at 30 mA in 8% SDS-PAGE slab gel. TDE results examined genetic relationships by computing the genetic distance according to Aquadro and Avise's equation (1981).

RESULTS AND DISCUSSION

The character of subgenus *Drosophila* is that there are 3-4 egg filaments, the coronal fertilized spermatheca is twisted longitudinally, and finally the testis is long screwed shape. The black band on the back of the abdomen is narrow or interrupted in the middle. The ten species used in this study can be divided into *virilis* and *quinaria* sections. The *quinaria* species group belong in *quinaria* section is derived from *immigrans-Hirtodrosophila* radiation (Throckmorton, 1975).

According to 15 allozyme analysis results examining the genetic relationships of 10 species of Korean subgenus *Drosophila*, total of 18 loci were detected. From the 18 loci, *Mdh*-2, *Got* and *Me* showed no intraspecific variation. Particularly, *Ao* and *Fum* showed no interspecific variations being monomorphic. The pattern for each locus is shown in Table 1.

In starch gel electrophoresis, *Mdh* is divided into *Mdh*-1 that moved to anode and *Mdh*-2 that moved to cathode and 3 alleles were detected in *Mdh*-2. There were no intraspecific variations for *Mdh*-2, *Got*, *Me*, *G6pd* and *Adh*. Also, *Fum*, *Ao* and *Odh*-2 were monomorphic with no interspecific variation. Finally, *Aph*-1 and *Est*-1 showed the greatest number with ten alleles.

In order to ascertain the interspecific genetic relationships by allozyme, the result of genetic relationships (Rogers, 1972) based on Table 1 is shown in Table 2.

D. angularis and *D. brachynephros* showed the highest close genetic relationship with 0.854. *D. curvispina* and *D. unispina* also showed highly close relationship of 0.851. On the other hand, *D. nigromaculata* and *D. kuntzei* showed the lowest calculation of 0.421, showing a furthest relationship in genetic distance among the *quinaria* section. The average genetic similarity in the *quinaria* section was 0.626. From three species in *virilis* section, *D. tsigana* and *D. lacertosa* were closely related with 0.781 and the average was 0.632.

UPGMA (Sneath and Sokal, 1973) results based on the genetic similarity in Table 2 is shown in Fig. 1. *D. virilis, D. tsigana*, and *D. lacertosa* form one group and the remaining seven species form another group. In the seven species of *quinaria* section, *D. angularis, D. brachynephros, D. unispina* and *D. curvispina* form one subgroup and *D. kuntzei* and *D. takadai* form another subgroup. *D. nigromaculata* forms a different subgroup from them.

There have been a vast number of studies to inquire of the speciation at the molecular biological level using allozyme. Ayala et al. (1974) reported that speciation stages based on the genetic similarity obtained from inter or intra specific comparison of *willistoni* group were designated semispecies if the average genetic similarity was over 0.798 and sibling species if the average is over 0.587, and non-sibling species if the average was less than 0.299. Based on these theories, average genetic similarity of the 3 species, *D. angularis, D. brachynephros* and *D. unispina* in the

Table 1. Allelic frequencies at 18 loci in the 10 species of the subgenus *Drosophila*.

Gene \ Species		*vir.*	*tsi.*	*lac.*	*ang.*	*bra.*	*cur.*	*kun.*	*nig.*	*tak.*	*uni.*
Mdh-1	a	0.09						0.75	0.21	0.55	
	b					0.73					
	c	0.91					0.83	0.25	0.46	0.35	0.38
	d				1.00						
	e		0.20	0.18			0.14			0.10	0.63
	f					0.27			0.33		
Mdh-2	a				1.00	1.00	1.00	1.00		1.00	1.00
	b								1.00		
	c	1.00	1.00	1.00							
Got	a								1.00		
	b	1.00									
	c				1.00	1.00					
	d						1.00	1.00		1.00	1.00
	e		1.00	1.00							
Me	a	1.00	1.00	1.00							
	b				1.00	1.00	1.00	1.00	1.00	1.00	1.00
Aph-1	a					0.55					
	b				1.00	0.46			0.84		
	c								*0.16*		
	d						0.18				1.00
	e		0.38				0.09	1.00		0.91	
	f			1.00							
	g						0.18			0.09	
	h	1.00	1.00								
	i						0.14				
	j						0.41				
Aph-2	a	0.48									
	b	0.52									
	c					0.21		1.00			
	d					0.79			0.50		0.50
	e						1.00		0.50		0.50
	f		1.00	1.00	1.00					1.00	
Xdh	a	1.00	0.22		1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b		0.78	1.00							
Acph	a				0.75	0.88		1.00	1.00	0.75	
	b						0.79				1.00
	c	1.00	0.42	1.00							
	d				0.25	0.13				0.25	
	e		0.58								
	f						0.21				
α-*Gpdh*	a		1.00								
	b	1.00		1.00			0.42				
	c				1.00	1.00	0.58	1.00		1.00	1.00

Gene \ Species		*vir.*	*tsi.*	*lac.*	*ang.*	*bra.*	*cur.*	*kun.*	*nig.*	*tak.*	*uni.*
Odh-1	a	1.00	1.00	1.00							
	b				1.00	1.00	1.00		1.00	0.17	1.00
	c							1.00		0.83	
Odh-2	a	1.00	0.59	0.29							
	b								1.00		
	c		0.41	0.71							
	d				1.00	1.00	1.00	1.00		1.00	1.00
Ao	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G6pd	a	1.00	1.00								
	b				1.00	1.00			1.00		
	c									1.00	
	d						1.00	1.00			1.00
	e	1.00									
									1.00		
Adh	a	1.00									
	b				1.00	1.00	1.00				1.00
	c		1.00	1.00							
	d							1.00		1.00	
Sdh	a	*0.63*									
	b					0.46					0.25
	c				1.00	0.55	1.00	1.00	1.00	0.67	0.75
	d									0.33	
	e	0.38	0.38	0.67							
	f			0.33							
	g		0.63								
Fum	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Est-α	a		0.63	0.30				0.25	0.18	0.13	
	b			0.25							
	c	1.00	0.38	0.40				0.25	0.18	0.13	
	d			0.05							
	e								0.25	0.25	0.19
	f				0.26	0.18			0.29	0.38	
	g					0.35	0.58				0.39
	h				0.24	0.32		0.75		0.25	0.28
	i				0.34	0.15	0.42		0.28		0.15
	j				0.16						
Est-β	a						0.42				0.24
	b										0.34
	c				0.17	0.24	0.58				0.42
	d	1.00	1.00	1.00							
	e				0.39	0.30					
	f					0.31		1.00		0.56	
	g				0.24	0.16			1.00	0.44	
	h				0.20						

Abbreviations of species names are as follows: *vir.* = *D. virilis*; *tsi.* = *D. tsigana*; *lac.* = *D. lacertosa*; *ang.* = *D. angularis*; *bra.* = *D. brachynephros*; *cur.* = *D. curvispina*; *kun.* = *D. kuntzei*; *nig.* = *D. nigromaculata*; *tak.* = *D. takadai*; *uni.* = *D. unispina*.

Table 2. Rogers' genetic similarities among the ten species of the subgenus *Drosophila* species.

Species	*vir.*	*tsi.*	*lac.*	*ang.*	*bra.*	*cur.*	*kun.*	*nig.*	*tak.*
D. tsigana	0.541								
D. lacertosa	0.573	0.781							
D. angularis	0.265	0.301	0.274						
D. brachynephros	0.284	0.265	0.237	0.854					
D. curvispina	0.290	0.258	0.243	0.664	0.660				
D. kuntzei	0.250	0.238	0.198	0.580	0.597	0.628			
D. nigromaculata	0.323	0.299	0.218	0.599	0.577	0.479	0.421		
D. takadai	0.279	0.327	0.287	0.667	0.620	0.589	0.791	0.445	
D. unispina	0.273	0.251	0.225	0.672	0.717	0.851	0.643	0.476	0.615

The abbreviations of species names are the same as in Table 1.

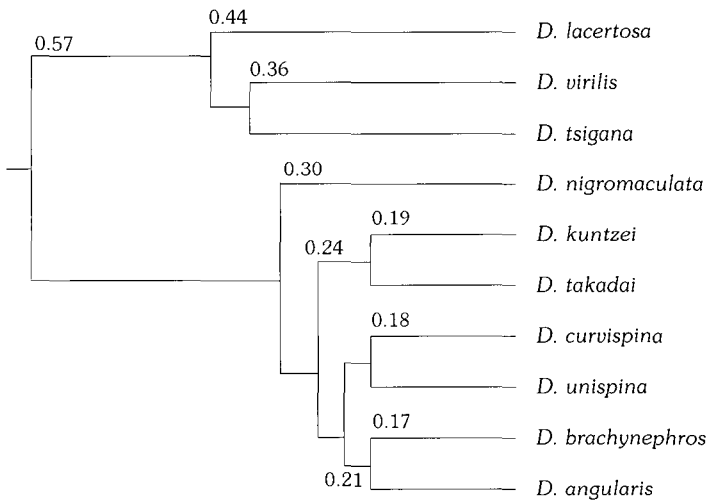

Fig. 1. UPGMA dendrogram showing the phylogenic relationships among the 10 species of the subgenus *Drosophila*, based on data of genetic similarity obtained by starch gel electrophoresis.

quinaria section was 0.744 as in the semispecies stage and *D. angularis* and *D. brachynephros* were the most similar species.

TDE were performed in order to study the relationship using soluble proteins. Approximately 100 or so protein spots appeared on the gel. Genetic distance between each species computed by Aquadro and Avise (1981) formula were as shown in Table 3. The results of TDE analysis showed that *D. curvispina* and *D. tsigana* had the furthest relationship with the genetic distance of 0.662. The relationship between *D. takadai* and *D. tsigana* was distant with the genetic distance of 0.616. On the other hand, the relationship between *D. angularis* and *D. brachynephros* was extremely close with the genetic distance of 0.168. In the *quinaria* species group, they were comparatively closely related with less than 0.322 of genetic distance.

Table 3. Genetic distances among the ten species of the subgenus *Drosophila* obtained by TDE.

Species	*vir.*	*tsi.*	*lac.*	*ang.*	*bra.*	*cur.*	*kun.*	*nig.*	*tak.*
D. tsigana	0.361								
D. lacertosa	0.456	0.431							
D. angularis	0.417	0.534	0.423						
D. brachynephros	0.432	0.536	0.475	0.168					
D. curvispina	0.516	0.662	0.554	0.207	0.216				
D. kuntzei	0.548	0.560	0.535	0.257	0.239	0.233			
D. nigromaculata	0.515	0.500	0.509	0.302	0.322	0.318	0.281		
D. takadai	0.542	0.616	0.527	0.251	0.215	0.201	0.192	0.316	
D. unispina	0.569	0.586	0.517	0.204	0.202	0.178	0.247	0.272	0.231

The abbreviations of species names are the same as in Table 1.

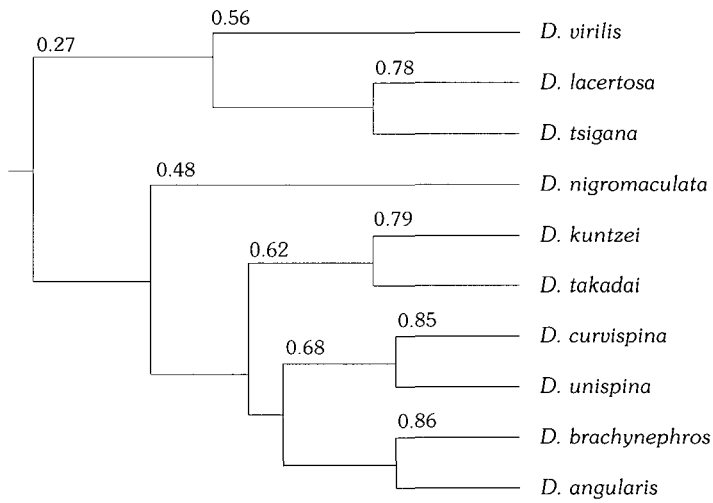

Fig. 2. UPGMA dendrogram showing the phylogenic relationships among the 10 species of the subgenus *Drosophila*, based on data of genetic distance obtained by two-dimensional electrophoresis. The numbers are branch length.

The results of UPGMA from the genetic distance in Table 3 are shown in Fig. 2.

In TDE analysis, the groups were divided into a group composed of the subgroup of *D. virilis* and *D. tsigana* and the subgroup of *D. lacertosa* and another group comprised of the remaining seven species as well. *D. angularis* and *D. brachynephros* were clustered at 0.168; *D. takadai* and *D. kuntzei* were clustered at 0.192; and the seven species in the *quinaria* species group were clustered at 0.302. Ohnishi et al. (1983) reported that the enzymatic variation from the allozyme and TDE analysis of *montium* subgroup was less than the variation from TDE analysis. Aquadro and Avise (1981) reported that the results of TDE and allozyme analysis on the interspecific relationships of eight species of rodent coincided with one another. Ohnishi et al. (1982) observed

similar relationships between those relationships obtained from TDE and allozyme analysis in the study of 15 species of *montium* subgroup in the subgenus *Sophophora*. In this study, the result of TDE revealed that the relationship between *D. angularis* and *D. brachynephros* was shown to be the closest in relation to one another. This coincided with the result of TDE analysis by Lee and Joo (1987). The result of the UPGMA analysis based on the TDE result was similar to the results of allozyme analysis. Okada (1988) stated that *virilis* section was more primitive than *quinaria* section based on the overall analysis of external shape, genitalia and reproductive organs, etc. of Drosophilidae. Theoretically, it can be hypothesized that *virilis* section was divided before *quinaria* section. From the seven species of *quinaria* section, it was hypothesized that *D. nigromaculata* was the first to be divided. Ten species of subgenus *Drosophila* can be divided into the 1st group of *D. virilis, D. tsigana* and *D. lacertosa* and the 2nd group of *D. angularis, D. brachynephros, D. curvispina, D. kuntzei, D. nigromaculata, D. takadai* and *D. unispina*. The 2nd group had four subgroups; the first subgroup clustered *D. angularis* and *D. brachynephros*, the second subgroup clustered *D. unispina* and *D. curvispina*, the third subgroup of *D. takadai* and *D. kuntzei* and the fourth subgroup of *D. nigromaculata* alone.

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동위효소와 수용성단백질 분석에 의한 한국 초파리아속 10종의 계통

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요 약

한국산 초파리과의 계통학적 연구의 일환으로, 초파리아속에 속하는 10종을 대상으로 동위효소와 수용성 단백질 분석을 실시하였다. 동위효소와 수용성 단백질 분석결과 *D.*(*D.*) *angularis*와 *D.*(*D.*) *brachynephros*가 유전학적으로 가장 가까운 종이었으며, *D.*(*D.*) *curvispina*와 *D.*(*D.*) *tsigana* 사이는 유전적 거리가 가장 먼 종으로 분석되었다. 그리고, 초파리아속 10종은 *D.*(*D.*) *virilis*, *D.*(*D.*) *tsigana*, *D.*(*D.*) *lacertosa*의 제1군과 *D.*(*D.*) *angularis*와 *D.*(*D.*) *brachynephros*, *D.*(*D.*) *unispina*와 *D.*(*D.*) *curvispina*, *D.*(*D.*) *takadai*와 *D.*(*D.*) *kuntzei*, 그리고 *D.*(*D.*) *nigromaculata*의 4개의 아군으로 이루어진 제2군으로 나눌 수 있다.