CHROMOSOMAL POLYMORPHISM IN NATURAL POPULATIONS OF *DROSOPHILA LUTESCENS*

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Chromosomal polymorphism in natural populations of various *Drosophila* species has been reported by many investigators (Patterson and Stone 1952; da Cunha 1955; Carson 1965; Dobzhansky 1970; Fisher 1973; and references therein). These studies, especially extensive studies on *Drosophila pseudoobscura* by Dobzhansky and his co-workers, have thrown light on the mechanisms by which chromosomal polymorphisms are maintained in natural populations. Chromosomal inversions protect gene complexes in chromosomes from dissociation through recombination. These gene complexes have been coadapted through long continued natural selection.

Drosophila lutescens is a member of *D. takahashii* subgroup which belongs to the *melanogaster* group of the subgenus *Sophophora* (Kikkawa and Peng 1938; Okada 1956; Bock and Wheeler 1972). The species is distributed from Korea to Japan excluding the Okinawa prefecture. Especially in the southern parts of Kyushu and Shikoku, this species and *D. takahashii*, both sibling species, seem to coexist.

The purpose of this study was to examine the chromosomal variability existing in natural populations of *D. lutescens*, and to obtain any information about the mechanisms maintaining chromosomal polymorphism in this species.

MATERIALS AND METHODS

The chromosome type of a male sampled from various populations was determined by crossing with females of a standard laboratory strain which has homologous gene arrangements, denoted as A, in each arm (Fukatami 1975). Two kinds of materials were used for the analysis of salivary gland chromosomes of this species. First, for six natural populations, Oshuka (Tohoku district), Kuroyama, Ogose, Yugawara and Kofu (Kanto), and Matsuyama (Shikoku) (see Fig. 1), a wild caught male fly or a single F₁ male from a wild, inseminated female, was mated with a few virgin females of the standard strain. These flies were collected simultaneously in 1973. Second, laboratory strains originating from twenty-two localities except Oshuka, Yugawara and Matsuyama among twenty-five localities shown in Fig. 1 were also employed. In the Kofu locality five samples were collected during 1968 to 1972 and in three localities, Todoroki (1968), Matsue (1968 and 1972) and Daisen (1971 and 1972), flies were sampled in two different seasons or years. In Niihama locality, flies were sampled at two stations

District	Locality	Abbreviation
Tohoku	Oshuku	OS
	Sakata	ST
	Sendai	SE
	Niitsu	NT
Kanto	Todoroki	TR
	Kuroyama	KY
	Ogose	OG
	Yugawara	YG
	Shimoda	SD
	Hachijo jima	HJ
	Kofu	KF
Hokuriku	Shiojiri	SJ
	Notoiida	NO
Kinki	Awazu	AZ
	Misedani	MS
	Ohara	OH
Chugoku	Daisen	DS
	Matsue	ME
	Okayama	OK
	Hiroshima	HR
Shikoku	Niihama	NH
	Ishizuchi yama	IS
	Matsuyama	MY
	Hirano	HN
Kyushu	Fukuoka	FK

Fig. 1. Collecting localities of *D. lutescens*.

Fig. 2. Inversions of four chromosome arms in the salivary gland chromosomes of *D. lutescens*. The open circles represent the basal ends of the arms.

about 1km apart from each other in 1972. In the remaining 17 localities flies were collected once during 1967 to 1972. Therefore, in total, thirty laboratory strains, were surveyed. For laboratory strains ten male flies were sampled at random from each

stock and mated individually with a few virgin females of the standard stock.

For the two kinds of materials described above, the salivary gland chromosomes of one larva, regardless of sex, were examined for each cross. No information with regard to the X chromosome therefore was obtained although a small median inversion was found in the X chromosome (Fukatami 1975). The gene arrangements of the second and third chromosomes were examined using lactic-acetic-orcein squash method. The small fourth chromosome carries no detectable inversions (Fukatami 1975). The various gene arrangements of this species are designated by the symbols as 2L A, 2L B, 2L C and so on in my previous paper (Fukatami 1975). They are shown in Fig. 2. On the left arm of chromosome 2 three inversions were discovered. One of them (2L D) was included in another inversion (2L C) and the other (2L B) was independent. Since two other structurally different chromosomes with two independent inversions (2L B and 2L C, or 2L B and 2L D) were detected, six kinds of gene arrangements including the standard one (2L A) were found in total. On the right arm of the chromosome 2, four inversions were found besides the standard arrangement (2R A) giving a total of five gene arrangements. For the V-sharped chromosome 3, four gene arrangements were observed in the left and five in the right arm as shown in Fig. 2. A total of twenty gene arrangements were observed in the four arms of the two chromosomes, among approximately 1000 flies scored.

RESULTS

The frequencies of different gene arrangements in the second and the third chromosomes of *D. lutescens* found in six natural populations are shown in Table 1. Each populations is highly polymorphic for four chromosome arms, 2L, 2R, 3L and 3R. All natural populations surveyed contain more than fifteen gene arrangements out of a possible twenty. The standard gene arrangements in each arm, A, were found in all localities except 3L A in Oshuku population. Some gene arrangements such as 2L B, 2L C, 2L D, 2L BC, 2R E, 3L B, 3L C, 3R B, 3R C and 3R D, were also observed in all localities from Tohoku to Shikoku. Some types of chromosomes were detected at only one locality, e.g., 2R B in Ogoe and 2R D in Matsuyama, and two gene arrangements, 3L BD and 3R BE, were not found in these six populations.

Since four natural populations, Kuroyama, Ogoe, Yugawara and Kofu, are located in Kanto district or its vicinity, data from these populations are pooled together. A homogeneity test of these four populations gave $\chi^2=39.88$, and $P>0.05$. Their mean frequencies are shown in the 7th column of Table 1.

In Table 1, a remarkable difference in frequencies of each gene arrangement was detected among three different districts, Tohoku, Kanto, and Shikoku. For instance, the frequency of 2L A was very high in Shikoku (83%) but a sudden decrease was seen in north-east part of Japan, Kanto (17%) and Tohoku (7%). Conversely, 2L BC has a frequency of 60% in Tohoku and 44% in Kanto, whereas its frequency is only 3% in Shikoku. As regards the right arm of the chromosome 2, the frequency of 2R A regularly decreases from south-west (Shikoku 94%) to north-east (Kanto 66% and Tohoku 42%). On the other hand, the increase in 2R E towards the north-east, 1% in Shikoku

Table 1. Frequency of the inversion of *D. lutescens* in natural populations

District	Shikoku		Kanto				Tohoku
Locality	Matsuyama	Yugawara	Ogose	Kuroyama	Kofu	Mean	Oshuku
Date	1973-IX	1973-VIII	1973-VI	1973-VI	1973-VI		1973-VII
Genomes	103	125	68	100	138	(431)	45
2L A	.83	.18	.18	.12	.20	.17	.07
B	.07	.11	.12	.07	.09	.09	.04
C	.07	.23	.22	.17	.23	.21	.16
D	.01	.02	.01	.04	.02	.03	.07
BC	.03	.44	.35	.54	.43	.44	.60
BD	0	.02	.12	.06	.04	.06	.07
2R A	.94	.62	.60	.67	.72	.66	.42
B	0	0	.01	0	0	.00	0
C	0	.10	.12	.11	.08	.10	.11
D	.05	0	0	0	0	0	0
E	.01	.28	.26	.22	.20	.24	.47
3L A	.44	.09	.06	.08	.07	.07	0
B	.22	.18	.12	.05	.06	.10	.02
C	.34	.73	.82	.87	.87	.82	.98
BD	0	0	0	0	0	0	0
3R A	.41	.20	.13	.27	.24	.21	.16
B	.02	.14	.23	.22	.20	.20	.33
C	.54	.30	.26	.15	.24	.24	.07
D	.03	.36	.37	.36	.32	.35	.44
BE	0	0	0	0	0	0	0

and 47% in Tohoku, compensates for the reduction of 2R A. Inversion 2R D was observed only in Shikoku with a very low frequency (5%). On the left arm of chromosome 3, 3L A was found in Shikoku (44%) and Kanto districts (7%), but was not detected in Tohoku. In north-eastern Japan, 3L C was the predominant arrangement reaching as high as 98% in Tohoku and 82% in Kanto, but only 34% in Shikoku. The frequency of 3L B increases towards south-west, being only 2% in Tohoku, 10% in Kanto and 22% in Shikoku. Regarding 3R, the frequency of 3R A plus 3R C was about 95% in Shikoku and 45% in Kanto. In Tohoku the frequencies can be as low as 23% and conversely 3R B and 3R D gave 77% in the pooled frequency. In Shikoku the sum of frequencies of both 3R B and 3R D was only 5%.

The results described above clearly suggest that a geographical cline exists from Tohoku to Shikoku at least in some gene arrangements. As thirty laboratory stocks were kept in mass in my laboratory for several months or a few years before examination, it is impossible to consider that these samples represent each local population as it was. However, it is possible that some information with respect to the clinal changes of each gene arrangement can be obtained. The thirty laboratory stocks cover twenty-two localities and can be grouped into six districts in Japan, e.g., Tohoku, Kanto,

Table 2. Average frequency of the inversions of *D. lutescens* in laboratory strains

District	Kyushu	Shikoku	Chugoku	Hokuriku Kinki	Kanto	Tohoku
Genomes	10	40	60	40	110	30
2L A	1.0	.95	.65	.50	.18	.17
B	0	.03	.15	.10	.12	.07
C	0	.03	.12	.38	.34	.37
D	0	0	0	.03	0	0
BC	0	0	.08	0	.36	.40
BD	0	0	0	0	0	0
2R A	.80	.98	.97	.95	.73	.73
B	0	0	0	0	0	0
C	0	0	0	.05	.05	.10
D	.20	.03	0	0	0	0
E	0	0	.03	0	.23	.17
3L A	.70	.53	.38	.33	.05	0
B	.20	.28	.23	.20	.16	0
C	.10	.20	.32	.48	.79	1.0
BD	0	0	.07	0	0	0
3R A	.40	.43	.48	.50	.25	.13
B	0	.08	.03	0	.16	.10
C	.60	.48	.42	.28	.27	.40
D	0	.03	.07	.15	.32	.37
BE	0	0	0	.08	0	0

Hokuriku-Kinki, Chugoku, Shikoku and Kyushu, from north-east to south-west.

The results, except for the geographically isolated Hachijo-jima population, is given in Table 2. Although the number of strains available is small for some districts the geographical cline found in Table 1 is also observed in Table 2. For instance, the frequency of 2L A increases gradually from north-east to south-west, 17% (Tohoku), 18% (Kanto), 50% (Hokuriku-Kinki), 65% (Chugoku) 95% (Shikoku) and 100% (Kyushu), respectively. The same tendency was found for the frequency of 3L A, e.g., 0% in Tohoku, 5% in Kanto, 33% in Hokuriku-Kinki, 38% in Chugoku, 53% in Shikoku and 70% in Kyushu. On the contrary, in frequencies of 3L C and 3R D a remarkable decrease from north-east to south-west was observed, e.g., for 3L C 100% (Tohoku), 79% (Kanto), 48% (Hokuriku-Kinki), 32% (Chugoku), 20% (Shikoku) and 10% (Kyushu), and for 3R D 37% in Tohoku, 32% in Kanto, 15% in Hokuriku-Kinki, 7% in Chugoku, 3% in Shikoku and 0% in Kyushu. It appears that the geographical clines found in Table 1 are more clearly confirmed in Table 2. The data of Tables 1 and 2 are summarized in Fig. 3. The frequencies of gene arrangements in natural populations shown as black columns are remarkably consistent with those of the laboratory stocks driven from the same district (white columns). It is clear that the frequencies of some gene arrangements, 2L A and 3L A, increase and others, 3L C and 3R D, decrease on a south-

Fig. 3. Diagram showing the frequencies of the inversions in four chromosome arms of *D. lutescens* in six districts of Japan. Black background indicates the frequency of the gene arrangements in natural populations. The white indicates the frequency of the inversions in laboratory strains.

west to north-east cline.

The main factor causing clinal changes of chromosomal variations in *D. lutescens* may attribute to temperature. An evidence that supports that assumption is shown in Fig. 4, illustrating the data of Hachijo-jima (open circle) which is not included in the calculation of frequencies given in Table 2. Hachijo-jima is a small island which belongs to Kanto district but lies about 280 km south of Tokyo and about 850 km east from Kyushu. This island is assumed to be almost southern bounds of the distribution area of this species. As seen in Fig. 4, the frequencies of 2L A, 3L A, 3L C, and 3R D in Hachijo-jima population are almost the same as those in Shikoku and Kyushu districts. It is reasonable to consider that the similarity between Hachijo-jima and south-west populations in chromosomal polymorphism is mainly due to the similarity in climatical conditions, especially in temperature, since these populations are located in warmer regions in Japan.

Nei (1972) proposed a formula to estimate the genetic distance between populations with respect to enzyme polymorphism. It may also be applied to chromosomal polymorphism in natural populations. In this case chromosomal arms and gene arrangements are used instead of enzyme loci and alleles, respectively. The estimates of chromosomal genetic distances are given in Table 3. It is clear that there are few differences in chromosomal composition between the four populations in Kanto district. The average genetic distance among Kanto populations is 0.018 ± 0.002 . On the other hand, the average genetic distance between any population in the Kanto district and

Fig. 4. Relation between the frequencies of the inversions in six districts and Hachijo-jima populations. Open circle showing the frequency of the inversions in Hachijo-jima population.

Table 3. Genetic distance (D by Nei 1972) estimated from inversion frequencies of the four arms of *D. lutescens* above diagonal among six natural populations. Below diagonal: between districts comparison

	Tohoku	Kanto				Shikoku
	Oshuku	Ogose	Kuroyama	Kofu	Yugawara	Natsuyama
OS		.0626	.0471	.0797	.0835	.8656
OG			.0244	.0141	.0149	.4313
KY	.0682			.0104	.0257	.4850
KF	$\pm .0072$	.0176	$\pm .0022$		.0165	.3729
YG						.3726
MY	.8656	.4154	$\pm .0233$

Fig. 5. Correlation between genetic distance and geographic distance.

Oshuku is 0.068 ± 0.007 , and the values between the former and Matsuyama is 0.415 ± 0.023 . Moreover, the genetic distance between Oshuku and Matsuyama populations is 0.866. The relationship between the genetic distance and the geographic distance is shown in Fig. 5. The geographic distances are estimated on the basis of railroad distances between localities. It is clear that relation between genetic distance and geographic distance is positive. The chromosomal composition in natural populations certainly differs between different districts. That is, the differences of chromosomal composition of the various populations are obviously proportional to the geographic distances which separate the localities.

The mean number of heterozygous inversion per individual in each district was estimated on the basis of the data in Table 1. The value of four populations in Kanto is 2.11 (Kuroyama), 2.35 (Ogose), 2.38 (Yugawara) and 2.12 (Kofu), while that in Oshuku and Matsuyama localities is 1.90 and 1.60, respectively. Therefore, the amount of chromosomal heterozygosity in the Kanto district seems to be higher than that in the Matsuyama or Oshuku populations.

To determine whether a correlation between the two arms of chromosome 2 or chromosome 3 exists in natural populations, the left and the right arms of the second chromosome and the two arms of the third chromosome are treated independently. Expected numbers of each chromosome type were calculated on the basis of random association between two arrangements. From the comparison between observed and expected numbers it is evident that the gene arrangements in the two arms of the second or the third chromosome are randomly associated in each of six populations. As shown in Table 4, chi-square values for these comparison gave a range from 0.10 to 3.64, resulting $P > 0.05$. In general, it can be said that crossing over between the left and the right arm may occur freely.

DISCUSSION

Many examples of geographical clines in chromosomal variations have been found in natural populations of *Drosophila* species (Dobzhansky 1970 and references therein). For instance, in *D. pseudoobscura* it is well known that clear gradients of the frequencies

Table 4. Association between two arms of the second or third chromosome of *D. lutescens* in six natural populations

Locality		Matsuyama		Yugawa		Ogose		Kuroyama		Kofu		Oshuku	
No. of genome		103		125		68		100		138		45	
2L	2R	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
A	A	79	80.0	14	13.7	9	7.2	11	8.0	23	19.7	2	1.3
A	others	6	4.9	8	8.3	3	4.8	1	3.9	4	7.5	1	1.7
others	A	17	16.0	61	62.4	31	33.1	55	56.2	76	78.2	15	16.5
others	others	1	1.9	42	40.5	25	22.6	33	31.7	35	42.7	27	25.5
χ^2		0.75		0.10		1.51		3.36		3.64		0.89	
3L	3R												
C	A	11	14.3	17	18.2	7	7.4	24	23.5	26	28.7	8	6.8
C	others	24	20.7	74	72.9	56	48.6	63	63.5	95	91.4	37	37.2
others	A	31	27.8	7	6.8	2	1.6	3	3.5	7	4.3	0	0.2
others	others	37	40.3	27	27.2	10	10.4	10	9.6	10	13.7	1	0.8
χ^2		1.93		0.10		1.26		0.10		3.09		0.46	

of various gene arrangements in the third chromosome exist. Other examples are the clinal changes from north to south in natural populations of *D. robusta* inhabiting the eastern United States (Carson 1958), and also in *D. subobscura* extending from northern Europe to Mediterranean and to Middle East (Krimbas 1964).

Krimbas (1964) concluded that the main selective agent determining clinal variations in *D. subobscura* was temperature. Corresponding to the clinal changes of the climatic factors, the change in the relative fitness of various genotypes may occur, resulting in the formation of the geographical cline. A similar case has been observed for elytral color pattern in a ladybeetle, *Harmonia exyridis*, in Japan (Komai 1956). He concluded that a climatic factor, probably temperature, was most responsible for the clinal change in the frequencies of alleles determining elytral color pattern. Experimental evidence for the differential effect of temperature on different chromosomal inversion types have been presented for *D. pseudoobscura*. Dobzhansky (1951) started four experimental cage populations with about 10% ST and 90% CH chromosomes derived from a natural population in California. At 25°C all came to the same equilibrium with the frequency of ST being 70%. However, when the same experiments were carried out at 16°C, the results were quite different showing no changes in the inversion frequencies. It is evident that the relative adaptive values of the chromosomal types were different at different temperatures, 16°C and 25°C.

In the present work the frequencies of some gene arrangements in the second and the third chromosomes of *D. lutescens* indicate that geographical clines exist from north-east to south-west in Japan. For instance, 2L A and 3L A arrangements increase from north-east to south-west and 3L C and 3R D decrease in frequency. In addition there is a marked similarity in inversion frequencies between Hachijo-jima and south-west populations, which are located in warmer parts of Japan. The average temperature

for a year in Oshuku is 9.4°C and that in Kofu, Kuroyama, Ogose and Yugawara in Kanto is 13.6°C, 13.1°C, 13.8°C and 15.2°C, respectively. Furthermore, the average temperature for a year in Matsuyama is 15.4°C and 18.1°C in Hachijo-jima. From the fact mentioned above, it is considered that temperature is a major factor causing the change of chromosomal variations in *D. lutescens*. Moreover, as shown in Table 4, it is likely that crossing over between the left and the right arms of the second and third chromosomes in this species may occur freely, therefore it seems that selection pressures operate on individual inversions independently.

According to collection records given in Bock and Wheeler (1972), Matsuyama is at the margin of the distribution of this species. Oshuku may also be considered as a northern marginal population though this species inhabits in Hokkaido. The degree of the chromosomal polymorphism is measured by the mean number of heterozygous inversion per individual. This value of polymorphism in Oshuku and Matsuyama localities is 1.90 and 1.60, respectively, but the average value of four populations in Kanto district is 2.24, ranging from 2.11 in Kuroyama to 2.38 in Yugawara. These results suggest that the "homoselection" theory of Carson (1959) which is based on studies of chromosomal polymorphism in *D. robusta*, may operate for populations of *D. lutescens*. In Japan, another example of chromosomal polymorphism has been reported by Yamaguchi *et al.* (1971) on *D. bifasciata*. They surveyed eleven natural population, extending from Hokkaido to Kyushu. Based on the number of heterozygous inversions per individual in each locality of the species, they conclude that populations located in the north-east of Japan are central for *D. bifasciata*, and a population in Kyushu is marginal. A possible reason for this difference in the location of the central populations of the two species, *D. lutescens* and *D. bifasciata*, may be due to the difference of habitats between these two species, since *D. lutescens* is a semi-domestic species and *D. bifasciata* is a wild one.

The formula for the genetic distance (D by Nei 1972) is applied to chromosomal polymorphism in natural populations of *D. lutescens*. The relationship between genetic distance and geographic distance is shown in Fig. 5. The genetic distance among populations which are not more than 700 km apart from each other is less than 0.09. On the other hand, the genetic distance is 0.45 among populations which are about 1000 km apart and is approximately 0.85 for populations separated from each other more than 1200 km. Certainly, the larger the geographic distance, the greater the genetic distance. The chromosomal genetic distance among Japanese natural populations of *D. bifasciata* is also calculated from data of Yamaguchi *et al.* (1971). The genetic distances obtained by pairwise combination of 11 populations vary ranging from 0.010, Kujusan in Kyushu district and Ishizuchisan in Shikoku, to 0.860, Kujusan and Daisetsusan in Hokkaido. In *D. bifasciata*, a correlation between the genetic distance and geographic distance is not seen, although it is clear that the chromosomal compositions of some populations are clearly different from each other.

SUMMARY

Chromosomal polymorphism in natural populations of *Drosophila lutescens*, a semi-domestic species, were studied. Chromosomal variations in six natural populations and thirty local strains maintained in laboratory culture were examined. In all populations remarkable inversion polymorphism was observed in four arms of the second and the third chromosomes. It was shown that there were clinal changes in the frequency of some gene arrangements (2L A, 3L A, 3L C and 3R D) from north-east to south-west in Japan. It is assumed that the main factor controlling the inversion frequencies is temperature.

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