Zoological Studies **33**(1): 29-33 (1994)

Zoological Studies

Mitochondrial DNA Variation in Natural Populations of *Drosophila immigrans*[1]

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(Accepted April 28, 1993)

Tadashi Aotsuka, Hwei-yu Chang, Megumi Aruga, Fei-Jann Lin and Osamu Kitagawa (1994) Mitochondrial DNA (mtDNA) variation in natural populations of *Drosophila immigrans*. *Zoological Studies* **33**(1): 29-33. The mitochondrial DNA restriction patterns of seven natural populations of *Drosophila immigrans* from North Vietnam, Taiwan, and Japan were analyzed. Using twelve restriction endonucleases we recognized a total of 18 mtDNA haplotypes. The mean number of nucleotide substitutions between mtDNA haplotypes (π_{ij}) was estimated to be from 0.002 to 0.016. The amount of mtDNA divergence within a population (π) was estimated to be from 0.0014 to 0.0077, while the net nucleotide differentiation among populations (d) ranged from -0.0004 to 0.0045. The estimated G_{st} value from mtDNA haplotype frequencies of each population was 0.205, indicating relatively uniform genetic constitution throughout the investigated *D. immigrans* populations. However, nucleotide diversity of the seven populations showed that the Taiwan and the southern Japan populations formed one cluster; the Sapporo population is slightly distanced from this cluster. The latitudinal systematic mtDNA differentiation among populations is discussed.

Key words: Intra-specific variation.

The *Drosophila immigrans* species subgroup of flies consists of about 15 species. Genetically and morphologically very similar, they are an excellent subject for evolutionary genetics study.

D. immigrans is known as one of the species that is widely distributed all over the world. The distribution of *D. immigrans* in tropical, subtropical, and temperate regions, implies that the species has the ability to adapt to a wide range of ecological fluctuations. Moreover, each *D. immigrans* population may adapt to its own local environmental conditions. If this is the case, some degree of genetic structuring among populations which coincides with ecological variations is expected. The study of genetic differentiation in *D. immigrans* natural populations leads towards an understanding of widely distributed species genetic structures.

Restriction analysis of mitochondrial DNA (mtDNA) has proven useful in genetic structuring of natural populations investigation (Avise et al. 1979, Saunders et al. 1986, DeSalle et al. 1987, Tamura et al. 1991). In this paper we report the results of the examination of the mtDNA variation in seven natural populations of *D. immigrans*.

MATERIALS AND METHODS

One hundred and twenty isofemale strains of *Drosophila immigrans* had been established from seven localities in North Vietnam, Taiwan, and Japan (Table 1, Fig. 1).

According to the procedures described by Tamura and Aotsuka (1988) mtDNA was isolated from adult flies of an isofemale strain. The twelve restriction enzymes that we used to recognize 6-bp nucleotide sequences (*Ban* III, *Bcl* II, *Bgl* II, *Eco*R I, *Eco*R V, *Hind* III, *Hpa* I, *Nsp* V, *Pvu* II, *Sac* I, *Sca* I and *Xba* I) detected mtDNA variations. Gel electrophoresis of digested mtDNA fragments

1. This is contribution no. 383 of the Institute of Zoology, Academia Sinica, ROC.

Table 1. Geographic origin and number of isofemale strains of *Drosophila immigrans* used in investigation

Country	Locality (year collected)	No. of Strains used in this study
North Vietnam	Hanoi (1987)	3
Taiwan	Chitou, Nantou (1990)	4
	Wulai, Taipei (1990)	35
Japan	Usuki, Ohita (1990)	8
	Tanga-Jima, Hyougo (1990)	17
	Todoroki, Tokyo (1990)	28
	Sapporo, Hokkaido (1990)	25

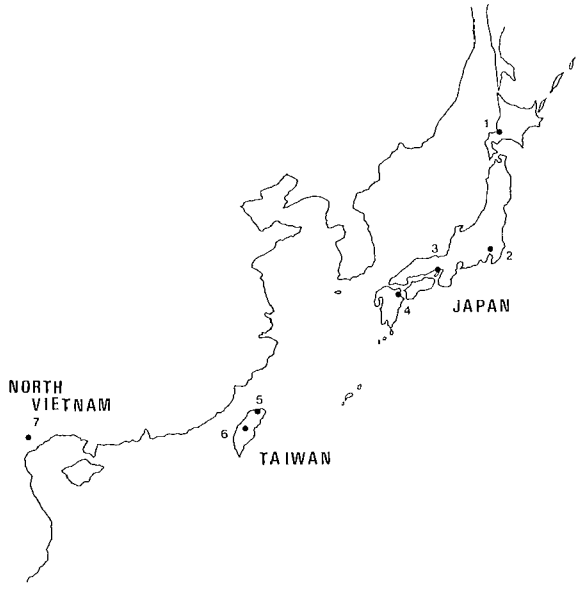

Fig. 1. Map showing the populations studied. 1. Sapporo, 2. Todoroki, 3. Tanga-Jima, 4. Usuki, 5. Wulai, 6. Chitou, 7. Hanoi.

were carried out with a 0.7% agarose slab gel in TPE (0.09M Tris-Phosphate, 0.002M EDTA) buffer (Sambrook et al. 1989). Restriction site maps were constructed by measuring the length of single-, double-, or triple-digested mtDNA fragments on the gel. *Sty* I digests of lambda DNA were adopted as a standard.

The mean number of nucleotide substitutions between mtDNA haplotypes (π_{ij}) was estimated by using formula #8 in Nei and Li (1979). The nucleotide diversity within a population (π) and the net nucleotide differences between the two populations (d) were estimated by using formula #18 in Nei and Tajima (1981) and formula #25 in Nei and Li (1979), respectively. A phylogenetic tree was reconstructed by using the unweighed pair-group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973).

RESULTS AND DISCUSSION

Using the twelve restriction enzymes we recognized a total of 45 restrcition sites in the 120 isofemale strains with an average of 38.0 sites mapped per isofemale strain. Among the recognition sites 15 were polymorphic defining a total of 18 mtDNA haplotypes (designated as IMM 1 to IMM 18). Table 2 shows the distribution of mtDNA haplotypes in natural populations of *D. immigrans*. All populations exhibited mtDNA restriction site polymorphism.

The mean number of nucleotide substitutions between haplotypes (π_{ij}) are illustrated in Table 3. Based on the data listed in Table 3, the 18 mtDNA haplotypes UPGMA tree shows several differentiated clusters (Fig. 1); but there was no tendency of haplotypes from the same country to cluster as a unit. This indicates that, on the mtDNA level, Japanese and Taiwanese populations of *D. immigrans* are unaffected by oceanic geographic isolation.

The extent of mtDNA divergence whthin a population (π) or among populations (d) were estimated with mtDNA haplotypes frequency (Table 2) and the nucleotide differentiation between mtDNA haplotypes (Table 3) are given in Table 4. Generally, the mtDNA divergence within a population was higher than that among populations. The average value of π was 0.0054, while d averaged only 0.0010. The relative magnitude of genetic differentiation among populations can be measured by G_{st} (Nei 1987). The estimated G_{st} from the mtDNA haplotype frequencies in each population was 0.205. Thus, only 20% of the total mtDNA diversity was attributed to population differentiation. Nucleotide diversity, nucleotide differentiation among populations and G_{st} suggest relatively uniform genetic constitution throughout the *D. immigrans* populations.

Still, the UPGMA dendrogram (Fig. 2) based on nucleotide differentiation among populations (d,

Table 2. Number of mtDNA haplotypes in each geographic population of *Drosophila immigrans*

mtDNA haplotype			Hanoi	Chitou	Wulai	Usuki	Tanga-Jima	Todoroki	Sapporo
IMM	1	aaaaaaaaaaaa	2	0	3	1	2	9	3
IMM	2	aaaaaaaaaaba	1	0	0	0	1	1	0
IMM	3	daaaaaaaaaaa	0	2	4	0	0	0	7
IMM	4	baaabaaaabaa	0	2	20	4	12	11	6
IMM	5	caaaaabaaaaa	0	0	0	0	0	0	8
IMM	6	aaaaaabaaaaa	0	0	0	1	2	3	1
IMM	7	baaacaaaabaa	0	0	0	0	0	0	0
IMM	8	aaaaaaaaabaa	0	0	1	0	0	1	0
IMM	9	aaaababaaaaa	0	0	0	0	0	1	0
IMM	10	daaaaaaaacaa	0	0	0	0	0	1	0
IMM	11	baaabaababaa	0	0	0	0	0	1	0
IMM	12	aaaaaacaaaab	0	0	0	1	0	0	0
IMM	13	baaadaaaabaa	0	0	0	1	0	0	0
IMM	14	baaabaaaaaaa	0	0	2	0	0	0	0
IMM	15	baaabaaabbaa	0	0	2	0	0	0	0
IMM	16	aaaabaaaabaa	0	0	1	0	0	0	0
IMM	17	aaaabaaaaaaa	0	0	1	0	0	0	0
IMM	18	daaaaaaaabaa	0	0	1	0	0	0	0
		Total	3	4	35	8	17	28	25

Haplotype is represented by the restrction patterns of 12 endonucleases: *Ban* III, *Bcl* I, *Bgl* II, *Eco*R I, *Eco*R V, *Hind* III, *Hpa* I, *Nsp* V, *Pvu* II, *Sac* I, *Sca* I, and *Xba* I (from left)

Table 3. Mean number of nucleotide substitutions per nucleotide site (δ) among mtDNA haplotypes in *Drosophila immigrans* (Figures on the diagonal are the number of restriction sites for each haplotype)

	IMM1	IMM2	IMM3	IMM4	IMM5	IMM6	IMM7	IMM8	IMM9	IMM10	IMM11	IMM12	IMM13	IMM14	IMM15	IMM16	IMM17	IMM18
IMM 1	39																	
IMM 2	0.002	40																
IMM 3	0.002	0.004	38															
IMM 4	0.009	0.011	0.012	37														
IMM 5	0.004	0.007	0.007	0.014	39													
IMM 6	0.002	0.004	0.004	0.011	0.002	40												
IMM 7	0.012	0.014	0.014	0.002	0.016	0.014	36											
IMM 8	0.002	0.004	0.004	0.007	0.007	0.004	0.009	40										
IMM 9	0.004	0.007	0.007	0.009	0.004	0.002	0.012	0.007	39									
IMM10	0.007	0.009	0.004	0.012	0.011	0.009	0.014	0.004	0.011	38								
IMM11	0.012	0.014	0.014	0.002	0.016	0.014	0.005	0.009	0.012	0.014	36							
IMM12	0.004	0.007	0.007	0.014	0.009	0.007	0.016	0.007	0.009	0.011	0.016	39						
IMM13	0.011	0.013	0.014	0.002	0.016	0.013	0.005	0.009	0.011	0.014	0.005	0.016	38					
IMM14	0.007	0.009	0.009	0.002	0.012	0.009	0.005	0.009	0.007	0.014	0.005	0.012	0.005	36				
IMM15	0.011	0.013	0.014	0.002	0.016	0.013	0.005	0.009	0.011	0.014	0.005	0.016	0.004	0.005	38			
IMM16	0.004	0.007	0.007	0.004	0.009	0.007	0.007	0.002	0.004	0.007	0.007	0.009	0.007	0.007	0.007	39		
IMM17	0.002	0.004	0.004	0.007	0.007	0.004	0.009	0.004	0.002	0.009	0.009	0.007	0.009	0.005	0.009	0.002	38	
IMM18	0.004	0.007	0.002	0.009	0.009	0.007	0.012	0.002	0.009	0.002	0.002	0.009	0.011	0.002	0.011	0.004	0.007	39

Table 4) revealed some interesting features. Two Taiwan populations (Chitou and Wulai) and two Japan populations (Usuki and Tanga-Jima) could not be distinguished by mtDNA variation. Todoroki, in central Japan, differed slightly from the Taiwan-Japan cluster; while Sapporo, in northern Japan, was distinct from other Japan and Taiwan populations. Although a small fraction of total mtDNA variation is attributed to inter-populational heterogeneity, latitudinal differentiation among populations is deserves consideration.

Gene flow among populations, which may

Table 4. MtDNA variation within populations (d, along the diagonal) and between populations (d_{xy}) of *Drosophila immigrans*

	Hanoi	Chitou	Wulai	Usuki	Tanga-Jima	Todoroki	Sapporo
Hanoi	0.0014						
Chitou	0.0018	0.0077					
Wulai	0.0045	−0.0003	0.0047				
Usuki	0.0032	0.0006	−0.0003	0.0017			
Tanga-Jima	0.0043	0.0001	−0.0001	−0.0004	0.0047		
Todoroki	0.0018	0.0005	0.0005	−0.0002	0.0003	0.0058	
Sapporo	0.0016	0.0006	0.0020	0.0009	0.0017	0.0005	0.0063

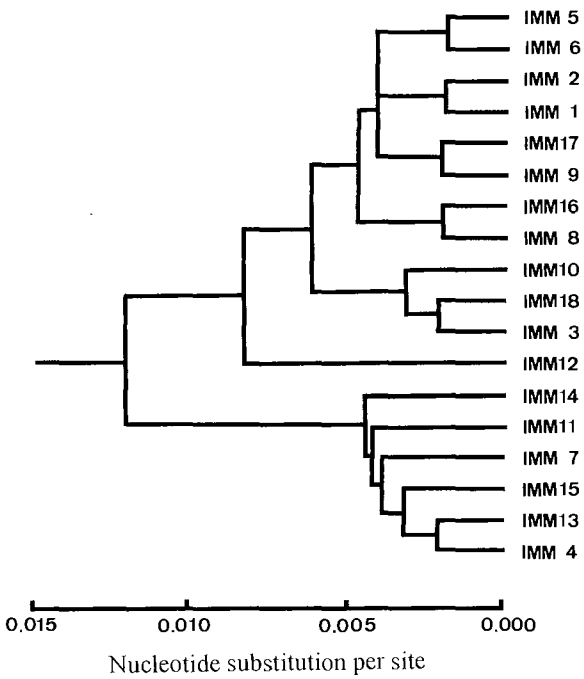

Fig. 2. UPGMA tree of the 18 mtDNA haplotypes of *Drosophila immigrans* based on the mean number of nucleotide substitutions per site (π_{ij}) among the haplotypes (Table 3).

occur at a high rate, may not be the only determinant for UPGMA tree topology, since the geographical distance between Japanese populations, even between Sapporo and Usuki, is much smaller than that between Japan and Taiwan.

The phylogenetic relationship shown by the UPGMA tree may imply that the genetic differentiation among populations is caused by climatic adaptation. In temperate regions temperature seems to be the most critical factor that affects survival of *Drosophila* (Izquierdo 1991). Natural populations of *Drosophila* in Japan locally adapt and overwinter. *D. immigrans* populations in northern or central Japan may have immigrated from southern areas. Among the immigrants, more adaptive flies could overwinter and establish stable populations. Despite successive migrations, ecological barriers have been strong enough to maintain some degree of genetic differentiation between northern, central, and southern populations in Japan. The lack of mtDNA differentiation among Taiwan and southern Japan populations indicates that a high degree of gene flow occurs with human activities (Slatkin 1989), and that there is no critical ecological barrier

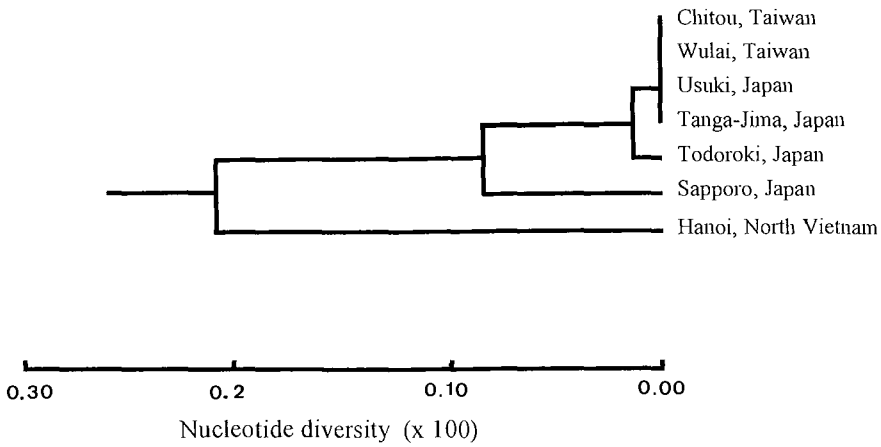

Fig. 3. UPGMA tree of the 7 geographic populations of *Drosophila immigrans* based on the net nucleotide diversity (d) among populations (Table 4).

for gene exchange between the two regions.

Alternatively, the severe coldness in northern or central Japan may be hard for *D. immigrans* to overcome. In these areas, the population fluctuates markedly from year to year. Consequently, genetic drift dramatically effects these populations. The observed mtDNA differentiation between northern or central Japan populations and southern Japan populations may be evidence of a temporal heterogeneity in natural *D. immigrans* populations.

The Hanoi population was very distantly related to all other Japan and Taiwan populations (Fig. 2). This suggests that *D. immigrans* in southeastern Asia has a different mtDNA polymorphism from those in Taiwan and Japan. Unfortunately, the available number of isofemale strains for the present study was too small to discuss the genetic structures for a wide range of southeastern Asia of *D. immigrans* populations.

More studies on the relationship between genetic variations as well as cold-resistance in natural populations of *D. immigrans* are needed. Still, our results show that mtDNA restriction analysis is a very sensitive method to examine genetic structure of natural population.

Acknowledgements: This work was supported with funds from the Overseas Scientific Expeditions in 1979 and 1981 (Grants No. 404149 and No. 56041049, respectively), the Ministry of Education, Science, and Culture of Japan to O. Kitagawa and the National Science Council of the Republic of China (Grant No. NSC74-0201-B001-44) to F. J. Lin and the National Science Council of the Republic of China (Grant No. NSC79-0409-B002-84) to H. Y. Chang.

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大果蠅(*Drosophila immigrans*)自然族群之粒線體DNA之變異

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由越南、臺灣及日本所採集的7個大果蠅(*Drosophila immigrans*)自然族群的果蠅，利用12種不同的限制酶(Restriction enzymes)分析其粒線體DNA，總共分析了18個粒線體DNA的單倍體型(Haplotypes)，每一核苷酸位置之核苷酸置換率(π_{ij})爲0.002～0.016，族群內粒線體DNA之分歧度(π)估計爲0.0014～0.0077，而族群與族群之間之分歧度(d)則爲−0.004～0.0045。由粒線體DNA單倍體型的頻度估計出每一個族群之遺傳分化度(G_{st})爲0.205。由UPGMA分析這七個族群之核苷酸分歧度顯示出，臺灣和日本南部的族群成爲一個群聚(cluster)，而日本北部的族群和上面這兩個地方的族群遺傳組成上有些差異，越南的族群其遺傳組成和臺灣和日本的族群差異較大。

關鍵詞：種內變異