Genetic differentiation in Japanese populations of *Drosophila simulans* and *D. melanogaster*. I. Allozyme polymorphisms

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ABSTRACT

Enzyme polymorphisms at 10 loci were studied both for *D. simulans* from the Japanese main island populations and Ogasawara population (the former has recently colonized and the latter has long been established) and for *D. melanogaster* from the main island and southern island populations including Ogasawara and Ryukyu Islands (these have been established). The proportion of polymorphic loci of *D. simulans* (32%) was lower than that of *D. melanogaster* (68%). However, a remarkable similarity in the average heterozygosities was found between *D. simulans* ($H=0.154$) and *D. melanogaster* ($H=0.156$). Differences in allele frequencies between years were small in both species. Among the 16 main island populations of *D. simulans* from Sapporo to Kagoshima, geographical uniformity of allozyme polymorphisms was found, but allele frequencies in the Ogasawara population were quite distinct from those of the main island populations. On the other hand, apparent geographical differentiation was found in *D. melanogaster* even among the 14 main island populations, as well as between the main island and Ogasawara populations.

1. INTRODUCTION

Drosophila simulans and *Drosophila melanogaster* have co-extensive wide range of distribution over the world in association with human habitations (Dobzhansky 1965, Bock and Wheeler 1972). These two sibling species are, however, largely rare or absent from Southeast Asia and West Pacific region (Bock and Wheeler 1972). Although *D. melanogaster* has been found in some localities including Japanese islands, *D. simulans* was reported only from the Ogasawara (Bonin) Island (Komai 1937, Kikkawa and Peng 1937, Okada 1956). Recently, however, *D. simulans* has immigrated into the main islands of Japan (Hokkaido, Honshu, Shikoku and Kyushu) and has been spreading rapidly (Watanabe and Kawanishi 1976, 1978). Population genetic studies of newly colonizing species, such as *D. simulans* in the main islands of Japan, may provide important information about the founder effect, processes of genetic differentiation, and the factors affecting the establishment of the differentiation.

The objective of the present study was to investigate the allozyme poly-

morphisms in newly colonizing populations of *D. simulans* in the main islands of Japan, comparing them with the long term established sympatric populations of *D. melanogaster*. The comparison demonstrated the geographical uniformity in the main island *D. simulans* populations, contrasting with considerable geographical divergence in *D. melanogaster* populations in the same region.

2. MATERIALS AND METHODS

Collection of flies

D. simulans and *D. melanogaster* were collected from various localities in the main islands of Japan, Ogasawara Islands, and Ryukyu Islands. Detailed information about the collections is summarized in Table 1 and Fig. 1. Flies around human habitations were collected using banana bait traps. In some

Fig. 1. Geographical distribution of sampling localities of *Drosophila simulans* (open circles) and *D. melanogaster* (darkened circles). Numbered localities correspond to those listed in Table 1.

Table 1. *Localities, collection dates and habitats of Japanese populations of Drosophila simulans and Drosophila melanogaster surveyed in the present study*

No.	Locality	(abbr.)	Species	Date (month/year)	Habitat
1	Sapporo I	(SPPI)	<i>simulans</i>	Sept./77	human habitation
2	Sapporo II	(SPPII)	<i>melanogaster</i>	Sept./77	beer brewery
3	Tsugaru	(TGR)	<i>melanogaster</i>	Oct./78	vineyard
4	Kogota	(KGT)	<i>melanogaster</i>	Oct./78	human habitation
5	Akayu	(AKY)	<i>melanogaster</i>	Oct./77, Oct./78	vineyard
6	Shiojiri	(SOJ)	<i>melanogaster</i>	Oct./77, Oct./78	vineyard
7	Omiya	(OMY)	<i>simulans, melanogaster</i>	Sept./77, Nov./78	human habitation
8	Kokubunji	(KKB)	<i>simulans</i>	Aug./77	human habitation
9	Setagaya	(STG)	<i>simulans</i>	Aug./77	human habitation
10	Katsunuma	(KTN)	<i>melanogaster</i>	Aug./77, Oct./78	vineyard
11	Oiso	(OIS)	<i>simulans, melanogaster</i>	Sept./77, Nov./78	human habitation
12	Mishima	(MSM)	<i>simulans</i>	Nov./77, Nov. 78	human habitation
13	Nagoya	(NGY)	<i>simulans, melanogaster</i>	Oct./78	human habitation
14	Shukugawa	(SKG)	<i>simulans, melanogaster</i>	Oct./77, Sept./78	human habitation
15	Habikino	(HBK)	<i>melanogaster</i>	Aug./78	vineyard
16	Okayama	(OKY)	<i>simulans, melanogaster</i>	Sept./78	vineyard
17	Matsuyama	(MTY)	<i>simulans</i>	Nov./77, Nov./78	human habitation
18	Ozu	(OZU)	<i>melanogaster</i>	Oct./77	human habitation
19	Kokura	(KKR)	<i>simulans</i>	Nov./77, Sept./78	human habitation
20	Harazuru	(HRZ)	<i>simulans</i>	Nov./77	persimmon yard
21	Bungomori	(BNG)	<i>simulans</i>	Nov./77	human habitation
22	Shimabara	(SMB)	<i>simulans</i>	Nov./77	human habitation
23	Izumi	(IZM)	<i>simulans, melanogaster</i>	Nov./77, Sept./78	human habitation
24	Kagoshima	(KGS)	<i>simulans</i>	Nov./77	human habitation
25	Ogasawara	(OGS)	<i>simulans, melanogaster</i>	Dec./77, July/78	human habitation
26	Okinawa	(OKN)	<i>melanogaster</i>	Feb./78	pineapple yard
27	Ishigaki	(ISG)	<i>melanogaster</i>	Feb./78	pineapple yard
28	Iriomote	(IRO)	<i>melanogaster</i>	Feb./78	human habitation

other habitats (mostly vineyards, beer breweries, and pineapple yards), flies were collected by sweeping. Flies of both species were sampled from eight geographic populations in two successive years, 1977 and 1978. In most cases, collected flies were frozen at -30°C for a few months, until electrophoresis.

Electrophoresis

Usually, wild caught females were used for electrophoresis. However, when the number of wild females was insufficient, wild caught males and F_1 females were also used. For both species, the following 10 enzymes (abbreviation in parentheses) were commonly assayed by horizontal thin layer gel

electrophoresis: acid phosphatase (ACPH), alcohol dehydrogenase (ADH), α -amylase (AMY), esterase-6 (EST-6), esterase-C (EST-C), glucose-6-phosphate dehydrogenase (G6PDH), α -glycerophosphate dehydrogenase (α -GPDH), malate dehydrogenase (MDH), malic enzyme (ME), and octanol dehydrogenase (ODH). Procedures for electrophoresis were the same as described by Fukutami (1977), with minor modifications in the buffer systems used in detecting EST-6 and EST-C. Conditions for electrophoresis and enzyme assays were nearly the same as described by Aotsuka (1980) and Ayala *et al.* (1972). Allozymes representing alleles at each locus were designated alphabetically by increasing order of mobility. Genetic distances between populations and between species were calculated according to Nei's formula (1972) from the allozyme allele frequencies.

Estimation of effective population size

From Omiya populations, male flies of *D. melanogaster* were collected in July of 1979 in order to estimate the effective size of the population based on the allelism rate of lethal genes (Kosuda *et al.* 1969). The number of loci and mutation rate of lethal genes were used following those estimated by Mukai and Yamaguchi (1974).

3. RESULTS

Electrophoretic variants

Table 2 shows the number of alleles at 10 enzyme loci examined, specific to each species, common to both species, and the total at each locus. In *D. simulans*, only four loci out of ten (*Est-C*, *Est-6*, *Acph*, and *Odh*) showed

Table 2. *Number of alleles at 10 enzyme loci in Drosophila simulans and Drosophila melanogaster*

Enzyme	<i>D. simulans</i>	<i>D. melanogaster</i>	Shared alleles	Total
ACPH	3	2	0	5
ADH	1	2	0	3
AMY	1	6	1	6
EST-6	3	4	3	4
EST-C	6	3	2	7
G6PDH	1	4	1	4
α -GPDH	1	2	1	2
MDH	1	2	1	2
ME	1	2	1	2
ODH	4	4	4	4
Total	22	31	14	39

polymorphisms, whereas all ten loci of *D. melanogaster* were polymorphic. At two loci, *Adh* and *Acph*, no alleles common to both species were detected. The number of alleles per locus was larger in *D. melanogaster* (3.1) than in *D. simulans* (2.2). Twentyfive (64%) out of 39 alleles in total were species specific, of which 17 (68%) were found in *D. melanogaster*. These results showed striking similarity to those of these two species reported by Berger (1970) in American populations. In the present study, some rare variants were newly found at the following loci: *Acph* of *D. simulans*, *G6pdh* in *D. melanogaster*, and *Odh* in both species.

Enzyme polymorphism of D. simulans

In *D. simulans*, twentyfive samples from 17 localities including Ogasawara, were studied (Table 3). Three out of ten loci (*Est-C*, *Est-6* and *Acph*), were found to be highly polymorphic throughout all samples. At the *Odh* locus, only Kyushu and Ogasawara populations were polymorphic. The average proportion of polymorphic loci was estimated to be 0.32 ± 0.01 . Average heterozygosity was 0.154 ± 0.002 for all *D. simulans* populations, although that was lower in the Ogasawara population, 0.125 in 1977 and 0.138 in 1978.

Most of the main island populations were found to be similar in allele frequencies at three polymorphic loci, *Est-C*, *Est-6* and *Acph*, with some exceptions found in the samples from Shukugawa and Kokura in 1977. On the other hand, the Ogasawara population (both in 1977 and 1978) were considerably different from the main island populations in the allele frequencies at the *Est-C* and *Acph* loci.

Rare alleles were observed at four loci, (*Est-C*, *Est-6*, *Acph* and *Odh*) in populations from both the main islands and Ogasawara. Among them the *Est-6^a* allele was found in 10 localities in the main islands, but not in the Ogasawara population. Other rare alleles, *Acph^a*, *Odh^b* and *Odh^a*, were also found only in some main island populations, but not in the Ogasawara population. In all eight populations surveyed, allele frequencies at each locus remained the same in two successive years with the exceptions of *Est-C* and *Est-6* in the Shukugawa population.

Enzyme polymorphism in D. melanogaster

The allele frequencies at the ten enzyme loci of *D. melanogaster* are shown for all samples in Table 4. Variants were found at all ten loci studied, although the degree of polymorphism varied among loci. The average proportion of polymorphic loci was 0.68 ± 0.02 , significantly higher than that of *D. simulans* (0.32 ± 0.01). In the Ogasawara population, however, the proportion of polymorphic loci was 0.4 in 1977 and 0.5 in 1978, lower than those of other populations. The average heterozygosity in *D. melanogaster* was 0.156 ± 0.006 , which was similar to that of *D. simulans* (0.154 ± 0.002). However, the range

Table 3. *Gene frequencies at 10 enzyme loci in Japanese populations of Drosophila simulans*

Locality	Est-C						Est-6				Acph				Odh			
	c	d	e	f	2*	(n)	b	c	d	(n)	a	b	d	(n)	b	c	2*	(n)
Hokkaido																		
1 SPP I	.464	.273	.145	.118	0	110	.377	.623	0	114	.377	.605	.018	114	0	1.0	0	114
Honshu																		
7 OMY'77	.490	.210	.183	.117	0	334	.298	.693	.009	342	.291	.709	0	340	0	1.0	0	340
8 OMY'78	.423	.315	.159	.103	0	390	.305	.629	.003	400	.268	.727	.005	400	0	1.0	0	400
9 KKB	.518	.156	.224	.102	0	294	.313	.687	0	300	.283	.717	0	300	0	1.0	0	302
11 OIS'77	.506	.296	.145	.053	0	318	.347	.647	.006	320	.406	.645	.007	290	0	1.0	0	286
12 MSM'77	.591	.214	.143	.052	0	384	.350	.647	.003	400	.348	.649	.003	400	0	1.0	0	400
13 NGY	.472	.205	.232	.091	0	220	.272	.728	0	228	.300	.696	.004	230	0	1.0	0	232
14 SKG'78	.504	.296	.156	.044	0	270	.323	.667	.010	300	.247	.753	0	320	0	1.0	0	316
16 OKY	.406	.261	.208	.125	0	264	.361	.540	.099	294	.298	.692	.010	302	0	1.0	0	302
Shikoku																		
17 MTY'77	.360	.310	.113	.217	0	300	.343	.657	0	312	.196	.804	0	312	0	1.0	0	314
19 KKR'77	.409	.341	.125	.120	.005	208	.367	.633	0	256	.125	.875	0	256	0	1.0	0	258
Kyushu																		
20 HRZ	.208	.450	.117	.225	0	120	.261	.739	0	230	.320	.680	0	228	.013	.987	0	232
21 BNG	.291	.362	.179	.168	0	340	.251	.732	.017	358	.349	.651	0	358	.006	.988	.006	358
22 IZM'77	.400	.165	.165	.270	0	248	.275	.725	0	316	.336	.664	0	318	.004	.996	0	256
23 IZM'78	.283	.287	.234	.193	.003	290	.232	.755	.013	298	.393	.607	0	300	.013	.987	0	300
24 KGS	.355	.168	.288	.189	0	386	.356	.636	.008	390	.431	.569	0	390	.023	.968	.009	222
Ogasawara Isls.																		
25 OGS'77	.322	.242	.272	.164	0	298	.340	.660	0	312	.414	.586	0	312	0	.997	.003	312
Mean (Total)	.396	.236	.232	.136	.001	(6316)	.310	.681	.009	(7672)	.339	.660	.002	(7708)	.003	.995	.002	(7594)

*: number of other alleles

(n): number of genes sampled

Table 4. Gene frequencies at 10 enzyme loci in Japanese populations of *Drosophila melanogaster*.
Explanations are same as Table 3

Locality	Adh			α -Gpdh			Est-6			Est-C			G6pdh		
	b	c	(n)	a	b	(n)	b	c	2*	(n)	a	b	a	b	(n)
Hokkaido															
2 SPPH	.198	.802	242	.157	.843	242	.880	.116	.004	242	.008	.934	.058	.237	.763
Honshu															
3 TGR	.500	.500	318	.169	.831	320	.862	.138	0	320	.059	.941	0	.041	.959
4 KGT	.296	.704	294	.122	.878	294	.849	.151	0	292	.024	.962	.014	.163	.837
5 AKY-77	.407	.593	582	.084	.916	582	.899	.101	0	576	.036	.957	.007	.144	.853
AKY-78	.350	.650	400	.130	.870	400	.858	.142	0	288	.008	.992	0	.136	.864
6 SOJ-77	.305	.695	712	.283	.717	720	.690	.310	0	388	.034	.963	.013	.199	.762
SOJ-78	.393	.607	400	.238	.762	400	.729	.271	0	388	.045	.942	.013	.148	.852
7 OMY-77	.112	.888	232	.385	.615	234	.957	.026	.017	232	.009	.993	.032	.032	.968
OMY-78	.226	.774	252	.319	.681	254	.897	.095	.008	252	.012	.941	.047	.158	.842
10 KTN-77	.369	.631	306	.278	.722	306	.837	.163	0	424	.043	.917	.040	.267	.719
KTN-78	.396	.604	318	.239	.761	318	.846	.151	.003	318	.031	.944	.025	.212	.785
11 OIS-77	.161	.839	366	.375	.625	360	.873	.124	.003	372	.039	.961	0	.152	.836
OIS-78	.186	.814	280	.364	.636	280	.835	.161	.004	280	.010	.977	.013	.100	.900
13 NGY	.071	.929	450	.400	.600	450	.836	.162	.002	450	.013	.982	.005	.065	.924
14 SKG-77	.159	.841	82	.293	.707	82	.866	.134	0	82	.036	.916	.048	.060	.900
SKG-78	.268	.732	164	.311	.689	164	.902	.092	.006	164	.018	.958	.024	.121	.879
15 HBK	.234	.766	278	.225	.775	276	.816	.165	.019	266	.021	.975	.004	.121	.879
16 OKY	.083	.917	446	.297	.703	424	.881	.119	0	446	.007	.986	.007	.004	.924
Shikoku															
18 OZU	.131	.869	502	.267	.733	502	.923	.077	0	496	.006	.980	.014	.048	.934
Kyushu															
23 IZM-77	.083	.917	156	.263	.737	156	.937	.063	0	158	0	.994	.006	.025	.941
IZM-78	.016	.984	318	.296	.704	318	.962	.035	.003	318	.003	.973	.024	.007	.980
Ogasawara Isls.															
25 OGS-77	.606	.394	472	.245	.755	470	.580	.420	0	398	0	1.0	0	0	.921
OGS-78	.676	.324	306	.204	.796	304	.610	.390	0	308	0	1.0	0	0	.980
Ryukyu Isls.															
26 OKN	.657	.343	134	.157	.843	134	.553	.439	.008	130	.015	.963	.022	.469	.531
27 ISG	.827	.173	324	.086	.914	324	.606	.391	.003	322	.009	.957	.034	.292	.699
28 IRO	.914	.086	442	.079	.921	442	.691	.309	0	314	0	.952	.048	.168	.832
Mean (Total)	.332	.668	(8776)	.241	.759	(8756)	.814	.183	.003	(8332)	.019	.961	.020	.130	.855

(to be continued)

Table 4. (Continued)

Locality	Amy				Odh			Acph			Mdh			Me		
	d	e	f	3*	(n)	b	c	2*	(n)	c	e	(n)	a	b	a	(n)
Hokkaido	.012	.025	.942	.021	242	.017	.983	0	242	.079	.921	242	1.0	0	.996	.004
2 SPII																242
Honshu																
3 TGR	.021	.009	.940	.030	320	.150	.850	0	320	.004	.996	282	1.0	0	1.0	282
4 KGT	.010	.033	.889	.068	276	.058	.942	0	294	.002	.998	388	1.0	0	1.0	388
5 AKY'77	.017	.012	.937	.034	592	.093	.907	0	582	.002	.998	460	1.0	0	1.0	460
AKY'78	.039	.009	.876	.076	398	.045	.955	0	400	0	1.0	320	1.0	0	1.0	320
6 SOJ'77	.004	.060	.935	.001	712	.064	.936	0	700	0	1.0	480	1.0	0	1.0	480
SOJ'78	.012	.022	.958	.008	394	.083	.917	0	400	0	1.0	400	1.0	0	1.0	400
7 OMY'77	.099	.055	.811	.035	220	.004	.958	.038	236	0	1.0	94	1.0	0	1.0	94
8 OMY'78	.060	.046	.841	.053	252	.016	.980	.004	258	0	1.0	96	1.0	0	1.0	96
10 KTN'77	0	.063	.937	0	302	.057	.943	0	424	0	1.0	216	1.0	0	.995	.005
KTN'78	.009	.038	.953	0	318	.070	.930	0	316	.009	.991	320	1.0	0	.994	.006
11 OIS'77	.015	.112	.843	.030	330	.046	.954	0	390	.015	.985	206	1.0	0	1.0	206
OIS'78	.033	.072	.870	.025	272	.021	.979	0	280	0	1.0	96	1.0	0	1.0	96
13 NGY	.021	.007	.850	.122	434	.009	.991	0	450	0	1.0	392	1.0	0	1.0	392
14 SKG'77	.046	.015	.833	.106	66	0	1.0	0	84	0	1.0	50	1.0	0	1.0	50
SKG'78	.037	.019	.907	.037	162	.024	.976	0	164	0	1.0	96	1.0	0	1.0	96
15 HBK	.002	.015	.892	.091	270	.044	.956	0	276	0	1.0	320	1.0	0	1.0	320
16 OKY	.013	.005	.867	.115	426	.014	.986	0	442	0	1.0	400	1.0	0	1.0	400
Shikoku																
18 OZU	.078	.028	.872	.022	320	.024	.976	0	500	.006	.994	320	1.0	0	1.0	320
Kyushu																
23 IZM'77	.020	.048	.878	.054	154	.013	.968	.019	158	0	1.0	98	1.0	0	1.0	98
IZM'78	.031	0	.869	.100	316	0	1.0	0	318	0	1.0	344	1.0	0	1.0	344
Ogasawara Isls.																
25 OGS'77	0	0	1.0	0	396	.003	.997	0	400	0	1.0	392	1.0	0	1.0	392
OGS'78	.003	.003	.988	.006	308	.007	.993	0	298	0	1.0	240	1.0	0	1.0	240
Ryukyu Isls.																
26 OKN	.016	.008	.976	0	124	.205	.795	0	132	.008	.992	134	.970	.030	1.0	134
27 ISG	.027	.007	.932	.034	308	.228	.772	0	324	0	1.0	368	.988	.012	1.0	368
28 IRO	0	.013	.915	.072	312	.281	.719	0	292	0	1.0	306	.994	.006	1.0	306
Mean (Total)	.024	.028	.904	.044	(8224)	.061	.937	.002	(3680)	.005	.995	(7060)	.997	.003	.999	(7060)

of average heterozygosities in the populations of *D. melanogaster* was wide (0.085–0.223). Low average heterozygosities were observed in Izumi (0.107 in 1977 and 0.085 in 1978), Okayama (0.125), and Ozu (0.123). On the other hand, the highest average heterozygosity was found in Okinawa island (0.223). The frequency of *Adh*^b (*Adh*^s by previous workers) allele was found to be the most variable among populations, from 0.914 (Iriomote) to 0.016 (Izumi): higher allele frequencies were observed in Ogasawara and Ryukyu Islands (Okinawa, Ishigaki and Iriomote) and in the northern part of the main islands, especially in vineyard populations. At three loci, α -*Gpdh*, *Est-6* and *G6pdh*, the allele frequencies varied from population to population, although the range of variation was not so wide as that of *Adh*. The frequency of α -*Gpdh*^a was higher in the central region of Honshu, especially in the populations near human habitation (Omiya, Oiso, and Nagoya). At both the *Est-6* and *G6pdh* loci, similar geographical distributions in allele frequencies were found: frequencies of *Est-6*^a and *G6pdh*^a were higher in the central part of Honshu (Shiojiri and Katsunuma) and southern islands (Ryukyu and Ogasawara). There were three loci, *Est-C*, *Amy* and *Odh*, in which one allele was in high frequency (80–90%) in most populations studied and relatively less divergent among populations. The three loci, *Acph*, *Mdh* and *Me*, were found to be monomorphic in most populations, although rare variants were detected in some populations; e.g. *Acph*^c comprised more than 7% of the Sapporo population.

In eight populations of *D. melanogaster* including Ogasawara, allele frequencies at ten loci were similar for the two successive years, 1977 and 1978, except for the Omiya population, in which frequencies of some alleles changed more than 10% between the two years at the *Adh* and *G6pdh* loci.

Effective population size of Omiya population in D. melanogaster

Effective population size was estimated using 248 males collected from the Omiya population in July of 1979, in order to determine whether random genetic drift might be a possible factor which caused the difference in allozyme frequencies between years described above. The percentage of each viability class, as adopted by Kosuda *et al.* (1969), was as follows: lethal, 20.6% (51/248); semi-lethal, 4.0% (10/248); subvital, 12.5% (31/248); normal, 62.5% (155/248); and super-vital, 0.4% (1/248). Allelism rate estimated by all possible combinations between 37 complete lethal genes was 2.1% (14/666). From the results, the effective population size of the Omiya population was estimated to be about 3,300.

Genetic differentiation in D. simulans and D. melanogaster

The mean genetic distances between years, between geographical popula-

Table 5. *The average genetic distance (Nei 1972) for various criteria, calculated from allele frequencies at 10 enzyme loci of Drosophila simulans and Drosophila melanogaster. Number in parentheses is combination number*

Criteria	<i>D. simulans</i>	<i>D. melanogaster</i>
overall	0.0091±0.0006 (300)	0.0244±0.0015 (325)
between years	0.0017±0.0006 (8)	0.0020±0.0004 (8)
within mainlands	0.0049±0.0002 (253)	0.0093±0.0005 (210)
between mainlands and Ogasawara Isls.	0.0327±0.0014 (46)	0.0354±0.0022 (42)
between mainlands and Ryukyu Isls.	—	0.0682±0.0034 (63)
between species.	0.6967±0.0020 (650)	

tions, and between species of *D. simulans* and *D. melanogaster* are shown in Table 5. In both species, genetic distances were small between years (0.0017 in *D. simulans* and 0.0020 in *D. melanogaster*) as compared with the distances among the geographical populations. Genetic distances among the geographical populations of *D. melanogaster* were generally larger than those found in *D. simulans*. Among the main island populations, the distance of *D. melanogaster* (0.0093) was about twice as large as that of *D. simulans* (0.0049). The average distances between the main island populations and the Ogasawara population were 0.0327 in *D. simulans* and 0.0354 in *D. melanogaster*. The largest genetic distance was found between the main islands and Ryukyu populations of *D. melanogaster* (0.0682). It is clear that, especially in *D. simulans*, the genetic distance within the main island populations were smaller than those between the main island and Ogasawara populations. The genetic distance between the two sibling species was 0.697 ± 0.002 , which was considerably larger than those among populations within each species (0.0017–0.0682).

4. DISCUSSION

The present study showed that the proportion of polymorphic loci (0.68) in *D. melanogaster* was larger than that (0.32) in *D. simulans*. In contrast, the average heterozygosity of *D. melanogaster* (0.156) was almost the same as that of *D. simulans* (0.154). These results were in accord with those of O'Brien and MacIntyre (1969), Berger (1970), Triantaphillidis *et al.* (1980) and Ohnishi *et al.* (1982). The *Est-C* locus was the most variable and responsible for the magnitude of average heterozygosity. At this locus, about 70–80% of individuals are heterozygous and the contribution of this locus to total average heterozygosity nearly corresponds to the total of the other loci. Authors who did not study the *Est-C* locus (Steiner *et al.* 1976, and Ohnishi *et al.* 1982) reported lower average heterozygosity in *D. simulans* (Table 6).

Table 6. Enzyme variation in several populations of *Drosophila simulans*

	Country	No. of loci	No. of alleles per locus	Proportion of polymorphic loci	Heterozygosity
O'Brien and MacIntyre (1969)*	USA (Columbia)	10	1.20	0.20	0.07
	USA (Manning)	10	1.00	0	0
Berger (1970)	USA	6	1.00	0	0
Kojima <i>et al.</i> (1970)**	USA (Texas)	18	2.17	0.556	0.162
Steiner <i>et al.</i> (1976)	USA (Hawaii)	15	1.60	0.40	0.073
	USA (Oahu)	15	1.33	0.333	0.073
Triantaphyllidis <i>et al.</i> (1980)**	Greece (Gorfu Is.)	11	2.09	0.455	0.174
Triantaphyllidis <i>et al.</i> (1982)**	Greece	6	2.13	0.50	0.172
Ohnishi <i>et al.</i> (1982)	Japan (Mishima)	24	—	0.29	0.067
	USA (mixed)	24	—	0.375	0.059
Present study**	Japan	10	1.64	0.32	0.154

*: Small number of isofemale lines studied

**: Studies including Est-C

The genetic distances were very small between years in both *D. melanogaster* and *D. simulans*. However, allele frequencies of some loci changed significantly between years. The largest change in allele frequency between years was observed at the *Est-C* locus of *D. simulans* in the Shukugawa population. The collection of that population in 1977 was the first record of its distribution in the district, and the number of flies sampled was small. This may be the cause of the unusual frequency in the 1977 sample. In the Omiya population of *D. melanogaster*, allele frequencies of *Adh* and *G6pdh* changed considerably between years, although the effective population size was estimated to be about 3,300. This value was in accord with those of Japanese *D. melanogaster* populations estimated earlier (Kosuda *et al.* 1969, Yamaguchi *et al.* 1980). The changes in allele frequencies between years may not have been caused by genetic drift, but may either be an experimental artifact caused by small sample size or mass migration.

Genetic differentiation and geographical distribution of D. simulans

D. simulans has successfully colonized the main islands of Japan in recent years and it is still spreading its distribution (Watanabe and Kawanishi 1976, 1978). In the course of this study, *D. simulans* was collected for the first time from some localities (Sapporo, Shukugawa and Okayama).

Very little genetic differentiation at the enzyme loci was revealed among the main island populations of *D. simulans*. Even between the most distant

populations (Sapporo vs. Kagoshima), the genetic distance was estimated as 0.0020, which is comparable to that between years at same locality (Table 5). This result suggests that populations of *D. simulans* in the Japanese main islands are probably from an ancestral population, although Ohnishi and Kawanishi (1981) suggested two different origins, at least. In the neighborhood of Japanese islands, *D. simulans* had been reported only in Ogasawara Islands before its invasion of the main islands of Japan. However, the possibility that the *D. simulans* now distributed all over the Japanese main islands derived from the Ogasawara Islands seems to be ruled out from the following fact clarified in the present study: rare alleles, such as *Est-6*^a, *Acph*^b and *Odh*^b, which were detected in the main island populations, had never been found in the Ogasawara population.

Table 7 shows allele frequencies of three polymorphic loci of *D. simulans* in various regions reported by previous authors. Number of major alleles at each locus and allele frequencies did not differ much among the regions. At both the *Est-6* and *Acph* loci similar allele frequencies were obtained all over. The low level of geographic differentiation at enzyme loci may be explained by species specific stable property or an essentially panmictic condition of *D. simulans* with great dispersal: it makes impossible to find the ancestor of the Japanese main island populations based on the allozyme data.

Genetic differentiation and geographic distribution of D. melanogaster

The present study shows some geographical differentiation of isozyme polymorphisms at several loci of *D. melanogaster*. Remarkable differentiation was found at *Adh* locus: *Adh*^b (*Adh*^s) frequency varied from 0.016 to 0.914. It has high frequencies in the southern islands, Ogasawara and Ryukyu Islands, and decreased frequencies in the main islands where the gradual decrease in *Adh*^b frequency from north to south was found, especially in the vineyard populations. In the populations near human habitations in main islands (Hokkaido, Honshu, Shikoku, and Kyushu) *Adh*^b frequency was regularly lower than those of vineyards in corresponding latitudes. This indicates that gene flow is not so great as to lose the diversity of *Adh* allele frequency, or that an environmental gradient is more responsible for the maintenance of the cline than gene flow.

The latitudinal *Adh* clines, decreasing *Adh*^s frequency with increasing latitude, were reported in United States (Johnson and Shaffer 1973, Voelker *et al.* 1978), in Europe and Africa (David 1982), in Australia (Oakesshott *et al.* 1982), and in Japan (Watanabe and Watanabe 1977, Yamaguchi *et al.* 1980). Following Watanabe and Watanabe (1977) and Yamaguchi *et al.* (1980), if we concern all populations including Ogasawara and Ryukyu where *Adh*^s is in particularly high frequency, the similar general latitudinal cline at *Adh* locus

Table 7. Allele frequencies of three polymorphic loci in several populations of *Drosophila simulans*

Enzyme	USA ¹⁾		USA ²⁾		Greece		Reunion ⁵⁾		Japan ⁶⁾	
	Texas	allele	Hawaii	Oahu	Gorfu island ³⁾	Mainland ⁴⁾	allele	allele	Main island	Ogasawara
Est-C	allele	allele			allele		allele	allele		
	f (2)	.185			1.07	.317	c	.083	c	.414
	f (3)	.247			1.13	.117	d	.365	d	.253
	f (4)	.296	—	—	1.17	.139	e	.356	e	.193
Est-6	f (6)	.277			1.25	.172	f	.185	f	.138
	f (1)	.285			1.30	.172				
Acph	f (2)	.224	.277	.333	S	.304	b	.440	b	.321
	f (3)	.730	.723	.667	F	.656	c	.513	c	.667
	f (3)	.730	.723	.667	F	.728	a	.120	a	.317
Acph	f (3)	.730	.723	.667	F	.728	b	.867	b	.681
	f (3)	.730	.723	.667	F	.728	b	.867	b	.681

- 1) Kojima *et al.* (1970)
- 2) Steiner *et al.* (1976)
- 3) Triantaphyllidis *et al.* (1980)
- 4) Triantaphyllidis *et al.* (1982)
- 5) Watada *et al.* (unpublished)
- 6) Present study

Table 8. *Frequencies of polymorphic isozyme alleles and linked inversions of D. melanogaster in Japan with correlations between them*

No.	Locality	Year	Isozyme		Inversion*		Isozyme		Inversion*	
			Adh ^b	α -Gpdh ^a	(2L)t	(3L)P	Est-6 ^b	(3R)P	(3L)P	(3R)P
2	Sapporo II	1977	.198	.157	.146	.013	.880	.050		
3	Tsugaru	1978	.500	.169	.160	.130	.862	.125		
4	Kogota	1978	.296	.122	.120	.050	.849	.085		
5	Akayu	1977	.407	.084	.225	.008	.899	.067		
	Akayu	1978	.350	.130	.245	.010	.858	.145		
6	Shiojiri	1977	.305	.283	.025	.017	.690	.283		
7	Omiya	1978	.226	.319	.087	.022	.897	.054		
10	Katsunuma	1977	.369	.278	.108	.017	.837	.254		
11	Oiso (Hiratsuka)	1978	.186	.364	.138	.014	.835	.065		
13	Nagoya (Nishiharu)	1978	.071	.400	.025	.015	.836	.070		
14	Shukugawa	1977	.159	.293	.156	0	.866	.125		
15	Habikino (Komagadani)	1978	.234	.225	.160	.071	.816	.076		
16	Okayama (Seto)	1978	.083	.297	.040	.030	.881	.025		
18	Ozu	1977	.131	.267	0	.004	.923	.017		
23	Izumi	1977	.083	.263	.022	0	.937	0		
	Izumi	1978	.016	.296	.005	0	.962	0		
25	Ogasawara (Chichijima)	1978	.676	.204	.230	.060	.610	.310		
	Correlation coefficient with inversion		.725**	-.642**		-.329		-.860***		

* Data from Inoue and Watanabe (1979) and Inoue *et al.* (1984)

** P<0.01

*** Correlation coefficient between Est-6^b and 3RP, P<0.001

was found. However, once attention is focused on Japanese main island populations (Hokkaido, Honshu, Shikoku and Kyushu) the opposite clinal pattern, increasing *Adh*^s with latitude, was found, especially in the vineyard populations. Although we have no data to explain this clinal variation, some factors such as microenvironmental variables, the frequency of linked inversion, *Ih*(2*L*)*t*, and migration might be responsible for the cline.

D. melanogaster is usually polymorphic in salivary gland chromosomes (Carson 1965, Ashburner and Lemeunier 1976, Inoue 1980). Linkage disequilibria between enzyme loci and closely linked inversions have been reported in *D. melanogaster* (Kojima *et al.* 1970, Mukai *et al.* 1971, Langley *et al.* 1974). It is also evident that some inversions exhibit clinal pattern over large geographical regions. Therefore, some authors have suggested that allozyme polymorphisms would be maintained by linked polymorphic inversions which were maintained by balancing selection (Mettler *et al.* 1977, Watanabe and Watanabe 1977). In this study, it is possible to compare the frequencies of allozymes with those of polymorphic inversions, because 17 populations of *D. melanogaster* studied were surveyed for chromosome arrangements at the same time by Inoue and Watanabe (1979) and Inoue *et al.* (1984). The degree of inversion polymorphism was low in the Shikoku and Kyushu population. The positive correlation between allele frequencies and linked inversion frequencies was significant in Japanese populations of *D. melanogaster* (Table 8). Gene frequencies of three polymorphic loci (*Adh*, α -*Gpdh* and *Est-6*) showed significant correlations with those linked inversions, although *Est-6* was not positively correlated with the closely linked inversion, *In*(3*L*)*P*, but strongly correlated with *In*(3*R*)*P*. These indicate that inversion polymorphisms are partly responsible for genetic differentiation of linked allozyme loci.

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