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Source: Zoological Science, 12(4) : 427-434

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.12.427

ZOOLOGICAL SCIENCE 12: 427–434 (1995)

Genetic Differentiation in Japanese Freshwater Crab, *Geothelphusa dehaani* (White): Isozyme Variation among Natural Populations in Kanagawa Prefecture and Tokyo

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ABSTRACT—In Kanagawa Prefecture and its surrounding area, two types of body color populations (**DA**: dark and **BL**: blue) of Japanese freshwater crab, *Geothelphusa dehaani*, are distributed parapatrically, suggesting some extent of genetic differentiation between them. In this study we examined the genetic variation of *G. dehaani* in Kanagawa Prefecture and Tokyo by means of electrophoretic analysis on 5 enzyme loci. The results clearly show the existence of substantial genetic differentiation among different color type populations. In addition, some extent of differentiation is also observed within the **BL** populations.

INTRODUCTION

Geothelphusa dehaani (White) is a freshwater crab inhabiting widely in the mainland of Japan (Honshu, Shikoku, Kyushu), Yakushima Island and Tanegashima Island [11]. Remarkable variation in body color has been observed [4, 5, 8, 13–15, 18] in natural populations of this crab. Chokki [4, 5] classified the body color types into three major categories, **DA** (dark), **BL**(blue) and **RE** (red), and examined the geographic distributions of these types in the northern and middle parts of Honshu. In the northern part of Honshu (Tohoku District) only **DA** crabs were distributed, whereas in the middle part of Honshu (Kanto and Tokai District) all three color types were distributed allopatrically or parapatrically. A distinct distribution pattern of different color type populations (**RE** and **BL**) was also observed in the southern part of Japan (Kagoshima Prefecture, Kyushu District), by Suzuki and Tsuda [14]. These observations lead us to think that some extent of genetic differentiation exists between different color type populations of *G. dehaani*.

Reports have been made on various morphological and electrophoretical studies intended to reveal genetic differentiation among local populations of *G. dehaani* [8, 13, 14]. However, little is yet known about detailed geographic or genetic structuring of natural populations in connection with body color polymorphism.

Recently, Suzuki [15] made extensive analyses of coloration and distribution of *G. dehaani* in the rivers of the Tanzawa Mountains (Kanagawa Prefecture) and found that populations of the two color types, **DA** and **BL**, were distributed parapatrically. At every river, **DA** crabs inhabit the upper reaches and **BL** crabs inhabit the lower reaches. Replacement of the distribution from one color type to the other was found to occur abruptly within a very narrow geographic range. Since the boundaries between the **DA** and **BL** populations at the Tanzawa Mountains were so clear, we planned to elucidate a detailed genetic or geographic structuring of *G. dehaani* populations in Kanagawa Prefecture and its surrounding area.

In the present study, using electrophoretic analysis, we studied genetic variation in natural populations of *G. dehaani* in Kanagawa Prefecture and Tokyo. The results clearly demonstrate the existence of substantial genetic differentiation associated with body color polymorphism.

MATERIALS AND METHODS

A total of 544 crabs were collected from 16 populations in Kanagawa Prefecture and 2 populations in Tokyo (Table 1 and Fig. 1). Crab's body color is known to vary depending on developmental stage and physiological conditions [4, 14, 15]. Suzuki [15] found that in Kanagawa Prefecture body color finally stabilized to either **DA** or **BL** when crabs developed more than about 20 mm in carapace width. Therefore, to characterize the populations with color types correctly and study the relationship between color polymorphism and isozyme variation, we tried to collect crabs larger than 20 mm in carapace. However, at collection sites where enough number of large crabs was not available, some small crabs were also collected to keep the sample size more than 20 for isozyme analysis.

Livers and muscles were extracted and homogenized with cold 40% sucrose solution. The supernatants of homogenates by centrifugation (12,000 rpm, 20 min.) were stored at −40°C before electrophoresis. Polyacrylamide gel [1] and horizontal starch gel electrophoresis [3] were performed for detection of isozyme variation at the five enzyme loci; acid phosphatase (*Acph*), amylase-3 (*Amy-3*), muscle esterase (*Est-M*), liver esterase (*Est-L*) and superoxide dismutase (*Sod*).

Accepted May 15, 1995
Received July 14, 1994
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TABLE 1. Collection information of *G. dehaani* examined in the present study

Population		n	% of crabs for each color type*					Population color type*
			DA	BL	TC	PB	GB	GP	
1	Hohkizawa	24	100.0						DA
2	Yumotodaira	31	100.0						DA
3	Shijyuhassegawa	22	100.0						DA
4	Hinata-3	27	85.2		7.4		7.4		DA
5	Hanamizugawa-1	34	97.1			2.9			DA
6	Hanamizugawa-2	39	100.0						DA
7	Suzukawa-1	30	83.4	6.7	3.3	3.3	3.3		DA'
8	Minamiasakawa	25	100.0						DA
9	Hinata-1	32	12.5	34.4	53.1				BL'
10	Hinata-4	24		66.7	33.3				BL
11	Suzukawa-3	28		46.4	53.6				BL
12	Hanamizugawa-3	43		53.5	34.9	11.6			BL
13	Hanamizugawa-4	38		65.7	21.1	7.9		5.3	BL
14	Kurihara	32		87.5	12.5				BL
15	Hatajyuku	26		65.4	34.6				BL
16	Moritogawa	32		84.4	15.6				BL
17	Tukuihama	32		81.2	18.8				BL
18	Yarimizu	25		28.0	64.0	8.0			BL

n: number of crabs collected. *For abbreviations of color types and population color types, see text for details.

RESULTS

The number of crabs collected and frequencies of different color types in each population are shown in Table 1. In all populations, smaller crabs of less than 20 mm in carapace width showed a variety of body colors that could be designated as **DA**, **BL**, **TC** (two-toned), **PB** (purple-brown), and **GP** (green-brown) according to Suzuki's classifications [15], whereas most of the larger crabs showed **DA** or **BL** body colors. Considering the observations that color types other than **DA** and **BL** are transitional in Kanagawa Prefecture and its surrounding region ([15] and our unpublished data), 16 out of 18 populations were characterized unambiguously as **DA** (7 populations: all or most crabs were **DA**, but none were **BL**) or **BL** (9 populations: high proportion of **BL** and **TC**, but no **DA** crabs) populations. In the remaining two populations, Nos. 7 and 9, the majority of crabs were **DA**, or **BL** and **TC**, but a few **BL** or **DA** crabs were contaminated, respectively. The color types of these two populations were then designated as **DA'** (No. 7) and **BL'** (No. 9).

Table 2 gives the allele frequencies, average heterozygosities and number of crabs examined for 5 different enzyme loci. Alleles at each locus are designated through numbering in increasing order of electrophoretic mobilities (Fig. 2). Most of the populations studied were polymorphic (i. e., the most common allele showed a frequency of less than 0.95) for all enzyme loci. Exceptional monomorphism was observed at the *Est-M* locus for **BL** populations Nos. 16, 17 and 18, and for this reason average heterozygosity tended to be lower in these three populations ($H=0.246$ to 0.332) than in the other populations. Although in many other populations the degree of polymorphism did not differ significantly, population No. 7 (color type **DA'**) showed an unusually high level of polymorphism. The value of average heterozygosity for this population was 0.542, whereas the unweighted mean of H in all populations was $0.387 \pm$S.E. 0.016.

The allele frequencies at the *Acph* locus were very similar throughout the populations. At the remaining four loci, however, remarkable differences in allele frequency were detected between **DA** and **BL** populations. At the *Est-L* locus, two alleles, *Est-L*^{2} and *Est-L*^{4}, were predominant in all the **DA** populations. Meanwhile, *Est-L*^{3} and *Est-L*^{5} were found in relatively high frequencies in all the **BL** populations. At the *Sod* locus, the allele Sod1 was the most common throughout the **BL** populations, ranging from 0.523 to 0.903, while this allele was rare or absent in most of the **DA** populations. The allelic differentiation between different color type populations was more pronounced at the *Amy-3* locus. Furthermore, at this locus, in addition to the considerable variation between the **DA** and **BL** populations, local differences in allele frequencies were also observed within the **BL** populations. Two alleles, *Amy-3*^{3} and *Amy-3*^{5}, were commonly predominant at the *Amy-3* locus in the **DA** populations, but a different allele, *Amy-3*^{2}, which was very rare in the **DA** populations, occurred in high frequencies in all **BL** populations. Moreover, in the **BL** populations of Nos. 16, 17 and 18, allele *Amy-3*^{4} was observed in high frequencies (0.419–0.629), whereas this allele was almost absent in other populations irrespective of body color type. A similar tendency was observed at the *Est-M* locus. In this case, **BL** populations Nos. 16, 17, and 18 were characterized by the fixation or near fixation of allele *Est-M*^{3}, which was found in

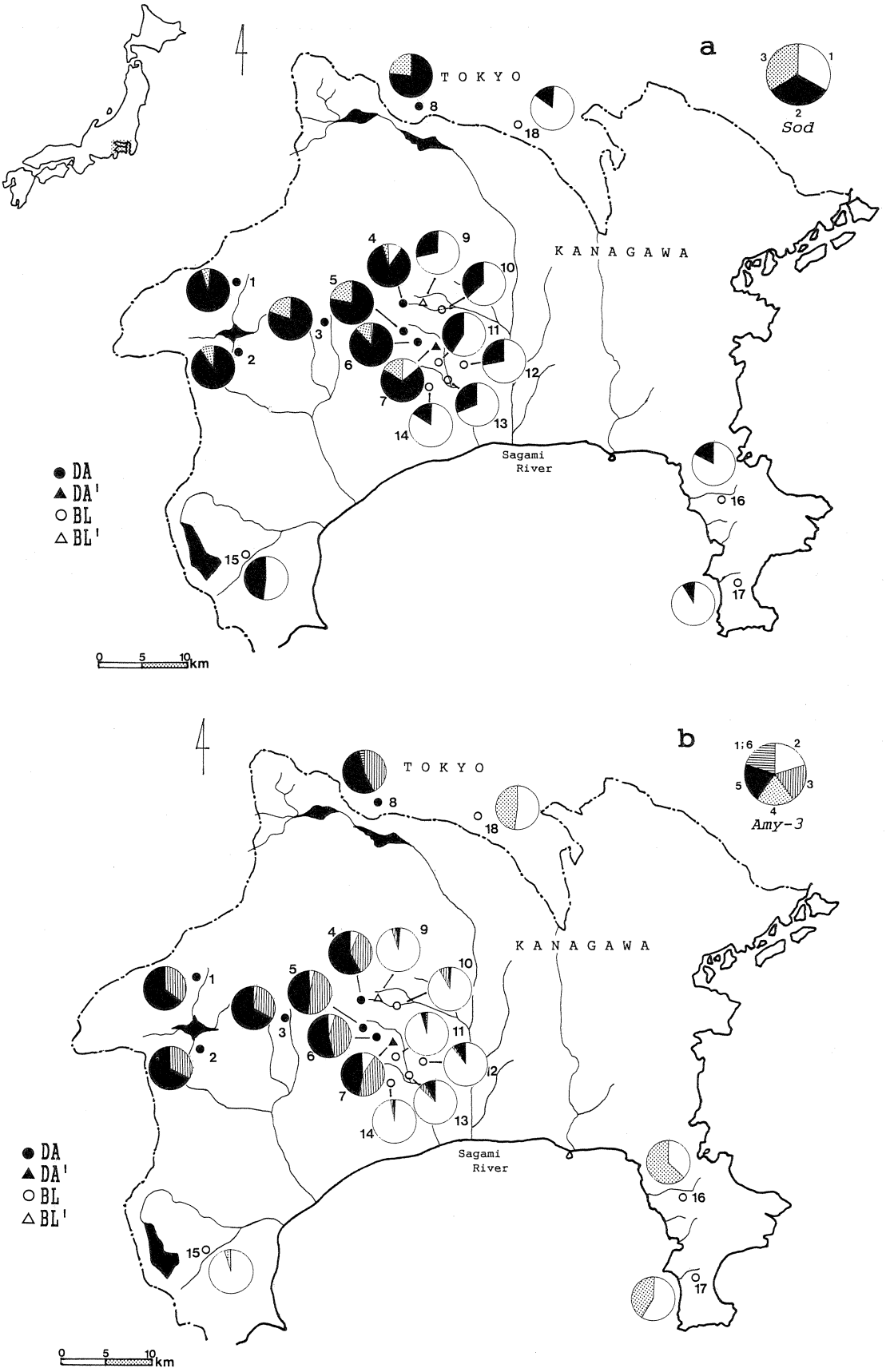

FIG. 1. Map showing the sampling populations of *Geothelphusa dehaani* with allele frequencies at the *Sod* (1a) and *Amy-3* (1b) loci. The numbers refer to populations as indicated in Table 1.

TABLE 2. Allele frequencies at 5 enzyme loci for 18 natural populations of *G. dehaani* in Kanagawa Prefecture and Tokyo

		DA Populations*								BL Populations									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Locus	Allele	(DA)	(DA)	(DA)	(DA)	(DA)	(DA)	(DA')	(DA)	(BL')	(BL)	(BL)	(BL)	(BL)	(BL)	(BL)	(BL)	(BL)	(BL)
Acph	*1*	0.150	0.146	0.325	0.063	0.074	0.175	0.155	0.130	0.174	0.184	0.339	0.219	0.159	0.000	0.088	0.103	0.000	0.000
	2	0.000	0.063	0.000	0.000	0.019	0.000	0.103	0.056	0.087	0.105	0.018	0.000	0.000	0.089	0.000	0.172	0.114	0.080
	3	0.850	0.791	0.675	0.937	0.907	0.825	0.725	0.814	0.739	0.711	0.625	0.781	0.841	0.911	0.912	0.725	0.886	0.920
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	n	20	24	20	24	27	20	29	27	23	19	28	32	22	28	17	29	22	25
Amy-3	*1*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.025	0.063	0.038	0.052	0.100	0.000	0.936	0.909	0.946	0.894	0.870	0.964	0.955	0.371	0.581	0.520
	3	0.354	0.333	0.300	0.354	0.481	0.414	0.433	0.426	0.032	0.068	0.000	0.015	0.043	0.018	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.629	0.419	0.480
	5	0.646	0.667	0.675	0.583	0.481	0.534	0.467	0.537	0.032	0.023	0.036	0.091	0.087	0.018	0.000	0.000	0.000	0.000
	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	n	24	24	20	24	27	29	30	27	31	22	28	33	23	28	22	31	31	25
Est-L	1	0.000	0.000	0.000	0.021	0.058	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.565	0.812	0.639	0.729	0.884	0.833	0.448	0.385	0.043	0.091	0.054	0.048	0.021	0.089	0.091	0.096	0.114	0.020
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.283	0.227	0.286	0.355	0.167	0.268	0.568	0.077	0.363	0.100
	4	0.435	0.188	0.361	0.229	0.058	0.111	0.466	0.596	0.109	0.045	0.036	0.016	0.083	0.089	0.114	0.115	0.023	0.060
	5	0.000	0.000	0.000	0.021	0.000	0.000	0.086	0.019	0.565	0.637	0.624	0.581	0.729	0.554	0.227	0.712	0.500	0.820
	n	23	24	18	24	26	27	29	26	23	22	28	31	24	28	22	26	22	25
Est-M	*1*	0.000	0.000	0.000	0.000	0.000	0.017	0.017	0.000	0.016	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.435	0.646	0.667	0.391	0.423	0.233	0.417	0.407	0.435	0.295	0.517	0.425	0.541	0.679	0.295	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.323	0.364	0.054	0.318	0.104	0.214	0.682	1.000	0.984	1.000
	4	0.543	0.354	0.333	0.609	0.519	0.550	0.417	0.519	0.210	0.341	0.357	0.212	0.292	0.107	0.023	0.000	0.016	0.000
	5	0.022	0.000	0.000	0.000	0.058	0.150	0.066	0.074	0.016	0.000	0.054	0.045	0.063	0.000	0.000	0.000	0.000	0.000
	6	0.000	0.000	0.000	0.000	0.000	0.050	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	n	23	24	21	23	26	30	30	27	31	22	28	33	24	28	22	29	31	17
Sod	*1*	0.000	0.000	0.000	0.104	0.000	0.017	0.133	0.000	0.710	0.636	0.589	0.719	0.687	0.833	0.523	0.823	0.903	0.840
	2	0.937	0.896	0.800	0.854	0.780	0.866	0.700	0.769	0.290	0.364	0.411	0.281	0.313	0.167	0.477	0.177	0.097	0.160
	3	0.063	0.104	0.200	0.042	0.220	0.117	0.167	0.231	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	n	24	24	20	24	25	30	30	26	31	22	28	32	24	27	22	31	31	25
	H	0.367	0.348	0.424	0.359	0.362	0.394	0.542	0.451	0.440	0.456	0.440	0.429	0.394	0.320	0.360	0.332	0.300	0.246

n: Number of crabs examined, H: Average heterozygosity. *Population No., (color type): See text and Table 1 for details.

moderate frequencies in other **BL** populations but was almost absent in the **DA** populations. In Figure 1a and 1b, graphic descriptions of frequency distributions at *Sod* and *Amy-3* loci are presented.

Based on the allele frequencies shown in Table 2, Nei's standard genetic distance, D [9], was estimated to evaluate genetic differentiation in natural populations of *G. dehaani* in relation to body color polymorphism (Table 3). Table 4 summarizes the mean values of D with standard errors by classifying the population comparisons into several categories. In this Table, **BL** populations are divided into **BL1** (population No. 16–18) and **BL2** (population No. 9–15) because of some extent of local differentiation as mentioned above. It is apparent from Table 4 that different color type populations, **DA** and **BL**, differ significantly in their allelic configurations. The mean value of D between **DA** and **BL** populations, 0.887 ± 0.027, is several ten times larger than those between populations of the same color type. Between **BL1** and **BL2** the value is estimated to be 0.266 ± 0.017, or that is several times larger than those within the **BL1** or **BL2** populations (0.028 and 0.057, respectively).

A dendrogram of genetic relatedness among the populations was constructed using the neighbor-joining method [10] from the matrix of genetic distance (Table 3) as shown in Figure 3. In the tree, the large two differentiated clusters of populations that correspond perfectly to the grouping of body colors are presented. The cluster of **BL** populations is further divided into two distinct subclusters, **BL1** and **BL2**. Within the **BL2** cluster, population No. 15 is somewhat distantly related from the other **BL2** populations.

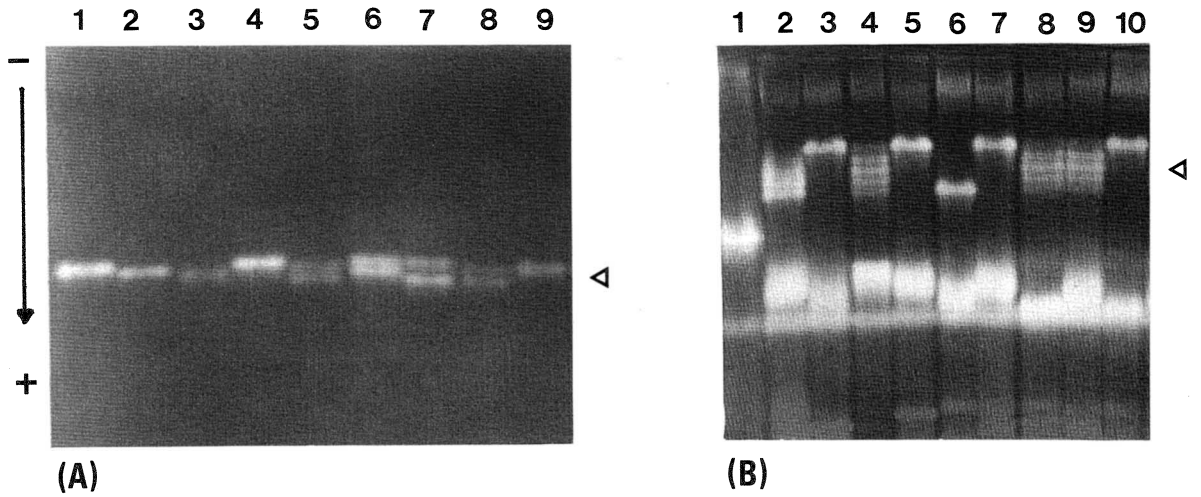

FIG. 2. Zymograms of *Amy-3* (A) and *Sod* (B) of *Geothelphusa dehaani*. Genotypes are (A) $Amy\text{-}3^2/Amy\text{-}3^2$; lanes 1 and 4, $Amy\text{-}3^3/Amy\text{-}3^3$; 2 and 9, $Amy\text{-}3^4/Amy\text{-}3^4$; 3, $Amy\text{-}3^2/Amy\text{-}3^3$; 6, $Amy\text{-}3^2/Amy\text{-}3^5$; 7, $Amy\text{-}3^3/Amy\text{-}3^5$; 5 and 8. (B) Sod^1/Sod^1; 3, 5, 7, and 10, Sod^1/Sod^2; 2, 4, 8 and 9, Sod^3/Sod^3; 1.

TABLE 3. Genetic distance among 18 populations of *G. dehaani* in Kanagawa Prefecture and Tokyo

	DA Populations*								BL Populations									
	1 (DA)	2 (DA)	3 (DA)	4 (DA)	5 (DA)	6 (DA)	7 (DA')	8 (DA)	9 (BL')	10 (BL)	11 (BL)	12 (BL)	13 (BL)	14 (BL)	15 (BL)	16 (BL)	17 (BL)	18 (BL)
2	0.034																	
3	0.035	0.020																
4	0.019	0.031	0.061															
5	0.057	0.033	0.074	0.023														
6	0.044	0.044	0.080	0.020	0.015													
7	0.025	0.061	0.047	0.044	0.075	0.072												
8	0.022	0.083	0.060	0.059	0.097	0.092	0.015											
9	0.802	0.828	0.805	0.704	0.821	0.845	0.595	0.786										
10	0.743	0.789	0.788	0.637	0.740	0.741	0.561	0.742	0.012									
11	0.714	0.735	0.693	0.639	0.743	0.744	0.557	0.724	0.034	0.038								
12	0.793	0.808	0.790	0.687	0.794	0.813	0.607	0.797	0.006	0.017	0.034							
13	0.694	0.721	0.702	0.608	0.715	0.746	0.525	0.681	0.023	0.033	0.025	0.024						
14	0.858	0.825	0.829	0.732	0.829	0.921	0.637	0.837	0.024	0.060	0.070	0.031	0.031					
15	0.768	0.797	0.808	0.689	0.780	0.793	0.623	0.779	0.085	0.101	0.173	0.083	0.169	0.125				
16	1.282	1.347	1.385	1.130	1.276	1.268	0.981	1.248	0.279	0.260	0.461	0.283	0.361	0.342	0.278			
17	1.278	1.325	1.409	1.067	1.193	1.215	0.982	1.261	0.189	0.189	0.371	0.184	0.289	0.229	0.142	0.041		
18	1.271	1.355	1.420	1.093	1.243	1.256	0.976	1.234	0.209	0.193	0.377	0.208	0.271	0.255	0.214	0.016	0.027	

* Population No., (color type): See text and Table 1 for details.

TABLE 4. Average genetic distance (D) between populations of *G. dehaani*

Comparisons	n	D±s.e.
DA	28	0.048±0.005
BL	45	0.153±0.018
BL1	3	0.028±0.006
BL2	21	0.057±0.010
BL1–BL2	21	0.266±0.017
DA–BL	80	0.887±0.027
All populations	153	0.517±0.035

n: Number of pairwise comparisons.
For abbreviations of populations, see text.

DISCUSSION

A species is usually divided in its distribution into local populations among which some genetic differentiation can be recognized by means of several methods of investigation. To understand the genetic basis of evolutionary process, and particularly of speciation, patterns of genetic structuring of local populations provide invaluable information [17]. However, dispersal and gene flow act as factors that override genetic differentiation among geographical populations [12]. Studies on genetic differentiation in Japanese natural populations of animals with a high dispersal capability, such as some species of *Drosophila*, have sometimes failed to detect clear

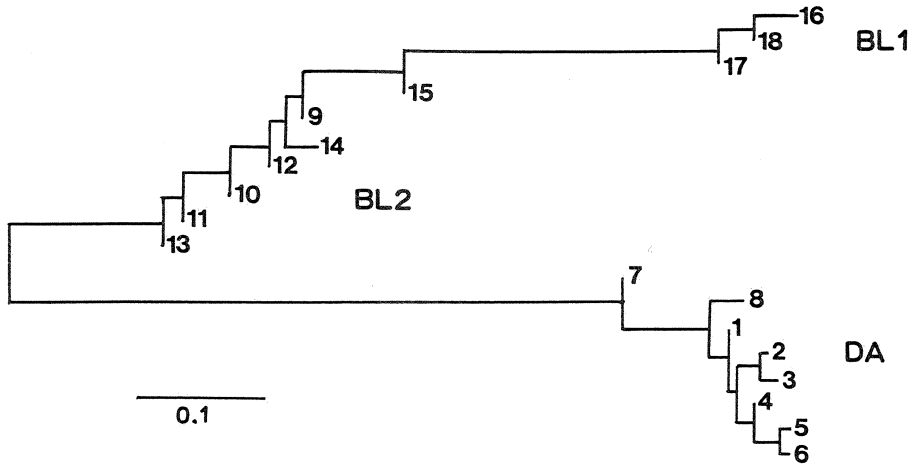

FIG. 3. Phylogenetic relationships among populations of *Geothelphusa dehaani*. The tree was constructed by the neighbor-joining method from the distance matrix of D (Table 3). The numbers and the abbreviations of color types are as in Table 1.

genetic structuring ([6, 16], but see [2]). The habitat of *G. dehaani* is restricted to small streams or swamps with clean freshwater. Juveniles of this crab develop from eggs without passing through a 'swimming' larval stage. In consideration of these characteristics, the dispersal capability of this crab is thought to be very low, and marked regional genetic differentiation, as suggested by the observation of body color polymorphism, is expected. Indeed, in the present study, using electrophoretic analysis, we found considerable differences in allele frequency among local populations of *G. dehaani* within a very restricted area, Kanagawa Prefecture and a part of Tokyo. Allozyme variation in Japanese natural populations of *G. dehaani* was also reported by Sugawara and Gamo [13] and Nakajima and Masuda [8]. Although these studies coincidentally suggested the existence of genetically differentiated local populations in relation to body color polymorphism, numbers of populations or isozyme loci examined were not sufficient to clarify the detailed genetic organization of *G. dehaani* in Japan.

Our study of genetic differentiation is based on the extensive analyses of body color polymorphism by Suzuki [15]. As shown in Table 1 and Figure 1, two types of color population, **DA** and **BL**, exist parapatrically in Kanagawa Prefecture, and the boundaries between these color types are very clear. Although the causative genetic or ecological mechanisms for body color polymorphism have not been completely resolved [7, 13], the pattern of distribution in Kanagawa Prefecture strongly suggests the existence of extensive genetic differentiation between the two color type populations. This prediction has turned to be correct. In Figures 1 and 3, substantial genetic differentiation among populations in complete association with color types is clearly indicated. Moreover, the **BL** populations can be divided into two further differentiated groups, **BL1** and **BL2** (Fig. 3), and the genetic division of **BL1** and **BL2** is associated with geographic location (Fig. 1b) The **BL1** populations (No. 16, 17 and 18), characterized by somewhat low heterozygosities due to a lack of variation at the *Est-M* locus and by a unique allele of the *Amy-3* locus (Table 2), lie east of Kanagawa Prefecture or southwest of Tokyo, while all the **BL2** populations lie west part of Kanagawa Prefecture (Fig. 1). Together with our unpublished data on *Amy-3* and *Sod* loci for several other populations in Kanagawa Prefecture, the presumable boundary between **BL1** and **BL2** is suggested to be Sagami River (Fig. 1). These results indicate that isozyme differentiation of *G. dehaani* occurs within a very narrow geographical range with a lack of association of morphological variation. Population No. 15 in **BL2** is positioned slightly distant from other **BL2** populations in the dendrogram (Fig. 3). The mean genetic distance between No. 15 and other **BL2** populations is 0.123 ± 0.015, whereas the value among **BL2** populations, including No. 15, is 0.057 ± 0.010 (Table 3, 4). Geographically, population No. 15 is the westernmost in this examination. The different allelic configuration observed in this population may indicate the occurrence of the 3rd genetically structured area of **BL** populations.

Population No. 7 is an exceptional case in the **DA** populations from where a small number of **BL** crabs were collected (Table 1), so we distinguished this population from the other **DA** populations, as **DA'**. Genetically, this population was found to be the most polymorphic ($H=0.542$) of all those examined (Table 2). In the dengrogram (Fig. 3), No. 7 is slightly differentiated from a cluster consisting of all other **DA** populations.

Since population No. 7 seems to locate on the boundary between the **DA** and **BL2** populations (Fig. 1), possible explanations for the coexistence of different color types, the high level of isozyme variability and slight genetic differentiaion from **DA** populations may be "contaminantion" of **BL** crabs into **DA** population or "hybridization" between **DA** and **BL** crabs. Examining the allelic configuration (Table 2) and the observed genotypes of isozyme loci for individual crabs (Table 5), some extent of gene flow by hybridization at population No. 7 is suggested. The alleles that exist in high frequencies in **BL2** populations, i. e., allele $Amy\text{-}3^2$, and Sod^1, tend to occur more frequently in population No. 7 than

in the other **DA** populations (Table 2). The exceptional **BL** crabs in this population, M4 and F1, did not necessarily show the genotypes typically observed in **BL2** populations (Table 5). At the *Amy-3* locus, for example, both crabs were heterozygous for alleles *Amy-3*^{2} and *Amy-3*^{5}. This genotype was very rare in **BL2** populations. At the *Est-L* locus, crabs M4 and F1 were homozygous for *Est-L*^{4} allele which was predominant in all **DA** populations but was rare in **BL2** populations (Table 2). Furthermore, among the **DA** crabs in population No. 7, some had unique genotypes. For example, F2, F13 and F15 crabs were heterozygous for alleles *Est-M*^{3} and *Est-M*^{4}. This genotype was never found in other **DA** populations because of complete lack of allele *Est-M*^{3}. From these observations, it is reasonable to think that the coexistence of different color types and the characteristic genetic structure at population No. 7 are due to hybridization between **DA** and **BL2** populations. However, the present data is not enough for any statistical test to confirm the hypothesis.

From population No. 9, several **DA** crabs were collected in the majority of **BL** crabs (Table 1). The genotypes of isozyme loci for individual crabs at this population are shown in Table 5. The exceptional **DA** crabs, F6, F12, F14 and F15, showed genotypes which were typically found in **BL** crabs. The coexistence of different color crabs in population No. 9 can, therefore, be also explained by hybridization. In this case, however, the extent of gene flow by hybridization seems to be less extensive than in population No. 7, because the allele frequencies and average heterozygosity of population No. 9 are fairly typical of **BL2** populations (Table 2, Fig. 3).

Although the value of genetic distance between **DA** and

TABLE 5. Isozyme genotypes (5 loci) for individual crabs collected from Population No. 7 and 9

Population 7							Population 9						
No.	Color	Genotypes					No.	Color	Genotypes				
		AC	AM	EL	EM	SO			AC	AM	EL	EM	SO
M 1	DA	23	33	24	44	12	M 1	TC	33	23	35	24	12
M 2	DA	33	25	24	24	12	M 2	BL	33	22	35	33	11
M 3	TC	33	55	24	22	22	M 3	BL	33	23	44	33	11
M 4	BL	13	25	44	44	12	M 4	BL	11	25	45	33	12
M 5	DA	11	35	24	22	33	M 5	TC	11	22	34	22	12
M 6	DA	33	35	22	24	23	M 6	BL	33	22	33	24	11
M 7	DA	33	22	22	24	23	M 7	TC	33	22	33	22	11
M 8	DA	13	55	22	44	22	M 8	TC	23	22	35	22	12
M 9	DA	33	55	24	22	23	M 9	TC	33	22	23	44	12
F 1	BL	—	25	44	24	12	M10	BL	33	22	55	15	12
F 2	PB	11	33	24	34	22	M12	TC	33	22	55	22	11
F 3	DA	34	35	25	24	22	F 1	TC	33	22	55	33	11
F 4	DA	33	55	55	22	22	F 2	BL	33	22	35	24	11
F 5	DA	23	33	22	44	22	F 3	BL	33	22	55	24	22
F 6	DA	33	55	44	24	12	F 4	BL	23	22	25	33	12
F 7	DA	33	35	24	46	23	F 5	BL	23	22	55	24	12
F 8	DA	33	33	—	44	23	F 6	DA	33	22	34	23	12
F 9	DA	33	33	44	24	22	F 7	TC	33	22	35	44	12
F 10	DA	33	35	44	22	23	F 8	TC	33	22	35	44	12
F 11	GB	33	35	24	24	12	F 9	TC	33	22	55	22	11
F 12	DA	22	55	24	12	23	F 10	TC	11	22	55	23	11
F 13	DA	23	35	22	34	22	F 11	BL	11	22	55	22	12
F 14	DA	33	35	24	24	22	F 12	DA	23	22	55	33	12
F 15	DA	33	35	25	34	12	F 13	TC	—	22	—	22	11
F 16	DA	13	35	24	24	22	F 14	DA	—	22	—	23	11
F 17	DA	11	33	44	22	22	F 15	DA	—	22	—	24	12
F 18	DA	33	23	25	25	22	F 16	TC	—	25	—	23	11
F 19	DA	33	55	44	24	22	F 17	TC	—	22	—	22	11
F 20	DA	23	23	44	45	22	F 18	TC	—	22	—	44	12
F 21	DA	33	35	22	55	13	F 19	TC	—	22	—	23	12
							F 20	TC	—	22	—	33	11

Individual crabs are represented by running numbers and M (male) or F (female). For abbreviations of color types, see text for details.

Isozyme loci are; AC *Acph*, AM *Amy-3*, EM *Est-M*, EL *Est-L*, SO *Sod*. —; not examined.

BL populations is very large compared to those within **DA** or **BL** populations (Table 4), and the presumable hybrid zone between different color types seems to be restricted within a very narrow range, it is still uncertain because of a small number of isozyme loci examined that these two color type populations are differentiated to the species level. Studies on a greater number of isozyme loci or on other genetic characteristics are needed. Whatever the taxonomic status is, however, considerable genetic divergence between local populations of *G. dehaani* within a very narrow geographic range is interesting for studies on the evolutionary forces of the speciation process.

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