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GENETIC VARIABILITY AND DIFFERENTIATION IN ISOZYMES IN *MNAIS* DAMSELFLIES OF FUKUOKA IN JAPAN (ZYGOPTERA: CALOPTERYGIDAE)

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To analyze the genetic differences, *M. pruinosa* and *M. nawai* were collected in 2 localities of Fukuoka Pref. Kyushu, Japan. In the Hisayama area, 2 forms of *M. pruinosa* ♂♂ occur, viz. orange winged f. *esakii* and clear winged f. *strigata*, and one ♀ form with clear wings f. *sieboldi*. In the Kami-ishigama area, *M. pruinosa* and *M. nawai* both occur and the forms of *M. pruinosa* are also f. *esakii*, f. *strigata* and f. *sieboldi*. 2 forms of *M. nawai* ♂♂ are also found, the orange winged f. *nawai* and the pale-orange winged f. *sahoi*, and one ♀ form with pale-orange wings f. ♀-*nawai*. The genetic differences among the samples collected in the areas were assessed by electrophoretic analysis. 21 protein loci of 10 proteins were analyzed by 5 % polyacrylamide gel electrophoresis; 2 of the 21 loci were monomorphic. The most variable strain (f. *strigata* of *M. pruinosa* from Hisayama) was highly polymorphic at 18 of the loci (P=0.857), with an average heterozygosity ($\bar{H}$) of 0.417. In all strains examined, the ranges of P and $\bar{H}$ values were 0.667-0.857 and 0.307-0.419, respectively. Nei's genetic distance (D) was used to distinguish the ♂♂ into 2 conspecific groups, the forms of *M. nawai* as group I, and the forms of *M. pruinosa* as group II. The ♀♀ together formed a third group. The genetic distance between groups I and II was close to, but above, the level of intraspecific variation. These results suggest that the ♂♂ f. *nawai* and f. *sahoi* of *M. nawai* should be categorized as a single sp., and that f. *esakii* and f. *strigata* of *M. pruinosa* should also be regarded as a single sp., but separate from *M. nawai*. All the ♀♀ showed a genetic relatedness indicating that they are within a single species (D ranging from 0.0595-0.0856), though they include the 2 different spp. of *M. nawai* (f. ♀-*nawai*) and *M. pruinosa* (f. *sieboldi*). They were the closest to f. *strigata* of *M. pruinosa* in the Kami-ishigama area, but the D value to group II was calculated as 0.1804, indicating a level of interspecific variation.

INTRODUCTION

Based on the comprehensive revision of the genus *Mnais*, ASAHINA (1975a, 1975b, 1976) defined three subspecies of Japanese *Mnais pruinosa*, viz. *pruinosa*, *nawai* and *costalis*, and various polymorphic forms in each subspecies. On the other hand, SUZUKI (1981, 1984) proposed four species of Japanese *Mnais*, *M. costalis*, *M. pruinosa*, *M. nawai* and *M. hiurai* (SUZUKI, 1985), based on a morphometric analysis and on geographical distribution. However, the validity of species is not easily demonstrated by this information alone. Among the species of Japanese *Mnais*, no genetic studies have been carried out. To know the genetic distances between forms of Japanese *Mnais*, can be helpful in distinguishing them from closely allied species.

Genetic analyses using protein electrophoresis have not previously been performed on dragonflies, except for a few studies. From the comparison of band patterns among the three species *Anax junius, Aeshna umbrosa* and *Libellula pulchella*, ANDERSON et al. (1970) suggested that protein analysis using acrylamide gel disc electrophoresis can be applied to problems involving closely related species within a single genus. KNOPF (1977) determined the genetic status of the genus *Gomphus* (Odonata: Gomphidae) using agarose gel electrophoresis. HARRISON et al. (1994) carried out this kind of study on *A. junius* and *Erythemis simplicicollis* larvae using starch gel electrophoresis and then documented enzymatic polymorphisms between and within species.

In the present study, we applied these electrophoretic techniques to the polymorphic forms of *Mnais* spp. collected from Fukuoka Prefecture, Kyushu, in Japan to determine the genetic variability in isozymes. In this paper, we use the genus--group name, *Mnais,* for convenience.

MATERIAL AND METHODS

Two species of adult *Mnais* were collected in two localities of Fukuoka Prefecture, Kyushu, Japan, in May 1992 (Tab. I).

(1) Hisayama area: A mountain stream, in the upper reaches of the Ino River, some 18 km northeast of Fukuoka City; the same area studied in previous reports (HIGASHI, 1976). In this area, two polymorphic forms of *M. pruinosa* males occur, orange- and hyaline-winged males (f. *esakii* and f. *strigata*; ASAHINA, 1976), and the female form with hyaline wings (f. *sieboldi*; SUZUKI, 1984). The abbreviations of these three forms, viz. f. *esakii*, f. *strigata* and f. *sieboldi*, are shown as 'ESh', 'STh' and 'SIh', respectively.

(2) Kami-ishigama area: The confluence of the main and tributary streams of the Muromi River located about 12 km from the mouth of the river. This river flows about 9 km to the west of Fukuoka City; the same area studied in previous reports (HIGASHI & UEDA, 1982). Two species of *Mnais* inhabit this area, i.e., *M. pruinosa* and *M. nawai*. The forms of *M. pruinosa* present are f. *esakii*, f. *strigata* and f. *sieboldi* (as in the Hisayama area). There are also two forms of *M. nawai* males present, the orange-winged f. *nawai* and the pale orange-winged f. *sahoi* (ASAHINA, 1976); also the female form with pale orange wings (f. *nawai*; YAMAMOTO, 1956). The abbreviations of f. *strigata* and f. *sieboldi* in *M. pruinosa* are shown as 'STk' and 'SIk', respectively. Only one male of *M.*

Table I
Abbreviations used for the strains of the three forms of *Mnais nawai* and the three forms of *M. pruinosa* at the two study localities

Study localities	*M. nawai*			*M. pruinosa*		
	Male		Female	Male		Female
	f. *nawai*	f. *sahoi*	f. *nawai*	f. *esakii*	f. *strigata*	f. *sieboldi*
Hisayama	–	–	–	ESh	STh	SIh
Kami-ishigama	NAk	SAk	NFk	–*	STk	SIk

* Present but not included in the electrophoresis because only one specimen was caught.

pruinosa f. *esakii* was collected in this area, therefore, it was not used in the electrophoresis. The abbreviations of f. *nawai*, f. *sahoi* and f. ♀-*nawai* are shown as 'NAk', 'SAk' and 'NFk', respectively. In this paper, we call each form from each population a 'strain'.

SAMPLE PREPARATION OF ENZYME EXTRACT AND ELECTROPHORESIS. – Fifty to 100 µl of a phosphate buffer saline (150 mM KCl, 3 mM Na_2HPO_4 $12H_2O$, 13 mM NaH_2PO_4 $2H_2O$, 5 mM EDTA) were injected into the thorax of adult *Mnais*. Haemolymph was collected from the thoracico--coxal joint of a hind leg into a micro tube (1.8 ml Eppendorf tubes) and placed in an ice-bath. For α--glycerol-phosphate dehydrogenase (α-Gpdh), the flight muscle was removed from the thorax after dissection and was homogenized in 50 µl of 5 mM 2-mercaptoethanol using a Teflon homogenizer in an ice-bath. For acid phosphatase (Acp) and leucine aminopeptidase (Lap), the gut was removed and homogenized in the same way as the flight muscle. Each haemolymph and tissue homogenate was centrifuged at 10,000 g for 10 minutes at 4°C. The resultant supernatants were then stored at -80 °C until required for electrophoresis. Five µl of the supernatant fluid was loaded into the sample slot. Electrophoresis was performed in a 5% horizontal polyacrylamide gel at 4°C. Electrophoretic techniques usually followed the method of TSUKAMOTO (1984, 1989). Buffer systems for the proteins examined are shown in Table II. Ten proteins from the eight strains derived from the six forms provided discrete bands and were selected for characterization. Six to 35 adult damselflies were used in each protein electrophoresis.

Table II
Proteins examined in electrophoretic analysis of 8 strains of *Mnais* spp.

Proteins	Abbreviation	Enzyme commision no.	Gel Buffer
Acid phosphatase	Acp	EC 3.1.3.2	0.1M Acetate, pH5.0
Adenylate kinase	Ak	EC 2.7.4.3	0.1M Tris-HCl, pH8.0
Aldehyde oxidase	Aox	EC 1.2.3.1	0.1M Tris-HCl, pH7.4
Glucose-6-phosphate dehydrogenase	G6pd	EC 1.1.1.49	0.1M Tris-HCl, pH8.5
α-Glycerolphosphate dehydrogenase	α-Gpdh	EC 1.1.99.5	0.1M Tris-HCl, pH7.4
Glucosephosphate isomerase	Gpi	EC 5.3.1.9	0.1M Tris-HCl, pH8.0
Leucine aminopeptidase	Lap	EC 3.4.11.1	0.2M Phosphate, pH7.4
Lactate dehydrogenase	Ldh	EC 1.1.1.27	0.1M Tris HCl, pH7.4
Phosphoglucomutase	Pgm	EC 2.7.5.1	0.1M Tris-HCl, pH8.0
Xanthine dehydrogenase	Xdh	EC 1.2.1.37	0.1M Tris-HCl, pH8.5

CALCULATIONS OF GENETIC DIVERSITY. – After the gel film had dried, the migration distance of each band was measured and the relative mobility was calculated as the R_f value (×100). The genetic variability in a strain was quantified by measuring the proportion of polymorphic loci (*P*) and the average heterozygosity per individual ($\bar{H}$). The average heterozygosity was calculated as

$$\bar{H} = 1 - \Sigma \bar{q}_i^2,$$

where q_i is the frequency of the i-th allele at a locus, and the average was calculated for all 21 loci examined. The genetic differences among the eight strains were estimated by calculating Nei's genetic distance (*D*) and genetic identity (*I*) (NEI, 1972).

RESULTS

DESCRIPTION OF PROTEIN VARIANTS

Considerable variation was observed in all loci examined in the eight strains except two, namely Gpi-2 and Ldh-2, which showed no variation. The genotype frequency and allele frequency variants of each protein are shown in Tables III and IV, respectively. Electrophoretic patterns were examined for their goodness-of-fit with regard to expectations based on the Hardy-Weinberg equilibrium (HWE) model. Chi-square (χ^2) - tests to fit the HWE showed that the distribution of the genotypes of most of the polymorphic proteins examined (Tab. III) were not significantly different from HWE. Detailed interpretations of protein variants are as follows:

ACID PHOSPHATASE (Acp). – Two loci appeared in all strains examined. The locus Acp-1 was clearly detected on the gel but was obtained from only a few samples in some strains; therefore, data on this locus were not included in the analysis. The Acp-2 locus showed high polymorphism in all strains examined. The males obtained from the Hisayama area showed a higher polymorphism than those from the Kami-ishigama area. ESh and STh appeared to have seven and eight genotypes (Tab. III) and to have been constructed by six and five alleles, respectively (Tab. IV). In the females, this locus also showed polymorphism and Acp^{60} was dominant.

ADENYLATE KINASE (Ak). – Ak-1 locus showed polymorphism in all strains (Tab. IV). The allele $Ak\text{-}1^{36}$ was dominant in the Kami-ishigama strains, except NAk and SAk, where $Ak\text{-}1^{33}$ was dominant; in the Hisayama strains, ESh, STh and SIh, $Ak\text{-}1^{33}$ allele was dominant. At the locus Ak-2, the $Ak\text{-}2^{14}$ allele showed monomorphism in all the male strains except STh, and there was a high frequency of this allele: 0.957 (Tab. IV). In all of the females, the two alleles of $Ak\text{-}2^{16}$ and $Ak\text{-}2^{14}$ appeared as a heterozygote at nearly equal frequency (Tab. III).

ALDEHYDE OXIDASE (Aox). – The locus Aox-1 showed polymorphism and the $Aox\text{-}1^{40}$ allele was dominant in all strains except STk and NFk, thus indicating monomorphism (Tab. IV). The highest polymorphism was observed in STh, which possessed six genotypes constructed by four alleles (Tabs III, IV). The Aox-2 locus had only one allele of $Aox\text{-}2^{16}$ in all strains except STk, where this allele was dominant; allele frequency was 0.850 (Tab. IV).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6pd). – Two protein loci appeared in

all strains, and each locus indicated a heterozygote (Tab. III). The alleles *G6pd-1*^{30} and *G6pd-2*^{13} were dominant in all strains at the first and the second locus, respectively, except for the two male Kami-ishigama strains. In contrast, in NAk and SAk, the alleles of *G6pd-1*^{33} and *G6pd-2*^{15} were dominant at each locus (Tab. IV).

α-GLYCEROLPHOSPHATE DEHYDROGENASE (α-Gpdh). – Three protein loci appeared in all strains. At the α-Gpdh-1 locus, the *α-Gpdh-1*^{68} allele was dominant in all strains except SIk and SIh, where the allele *α-Gpdh-1*^{65} was dominant (Tab. IV). The two male Kami-ishigama strains, NAk and SAk, had only one allele, *α-Gpdh-1*^{68} and *α-Gpdh-2*^{54} at α-Gpdh-1 locus and α-Gpdh-2, respectively. At the locus α-Gpdh-2, the remaining six strains had *α-Gpdh-2*^{51}, which was dominant, except for STh. The highest polymorphism was observed in STh which possessed five genotypes constructed by five alleles (Tabs III, IV). The locus α-Gpdh-3 had two alleles, *α-Gpdh-3*^{15} and *α-Gpdh-3*^{11}, indicating a heterozygote with nearly equal allele frequencies in all strains except STk, which showed monomorphism of the *α-Gpdh-3*^{11} allele.

GLUCOSEPHOSPHATE ISOMERASE (Gpi). – The locus Gpi-1 showed high polymorphism in all strains (Tab. IV). STh was especially polymorphic at this locus, possessing five genotypes constructed by four alleles (Tabs III, IV). The locus Gpi-2 was regarded as monomorphic in all strains because it had only one allele, *Gpi-2*^{14}.

LEUCINE AMINOPEPTIDASE (Lap). – Five loci appeared in all strains. The data on loci Lap-1 and Lap-5 were omitted in this study because there were only a few samples in some of these strains. The three other loci, Lap-2, Lap-3 and Lap-4, showed high polymorphism in all strains (Tab. IV). At the locus Lap-2, the *Lap-2*^{64} allele was dominant in all strains except NFk, where the frequency of this allele was very low (0.063) and the *Lap-2*^{68} allele was dominant. STh showed the highest polymorphism at this locus. At the locus Lap-3, the *Lap-3*^{46} allele was dominant in the female strains. At the locus Lap-4, none of the male strains had the *Lap-4*^{23} allele, although this allele was dominant in the females.

LACTATE DEHYDROGENASE (Ldh). – The Ldh-1 locus showed a polymorphism and the *Ldh-1*^{38} allele showed the highest frequency in all strains (Tab. IV). Only the male strains had the *Ldh-1*^{40} allele. The locus Ldh-2 was regarded as monomorphic in all strains because it has only one allele, *Ldh-2*^{15}.

PHOSPHOGLUCOMUTASE (Pgm). – The Pgm-1 locus showed a polymorphism and possessed at least two alleles, *Pgm-1*^{33} and *Pgm-1*^{30}, in all strains (Tab. IV). Three strains, STk, ESh and STh, had one more allele, *Pgm-1*^{36}, indicating a heterozygote constructed by *Pgm-1*^{36} and *Pgm-1*^{33} (Tab. III). At the locus Pgm-2, a heterozygote constructed by the alleles, *Pgm-2*^{15} and *Pgm-2*^{12}, was obtained only from the males. In contrast, the females showed a monomorphism having the *Pgm-2*^{15} allele alone.

XANTHINE DEHYDROGENASE (Xdh). – At the locus Xdh-1, the allele *Xdh-1*^{31} was extremely high in frequency in all of the male strains (Tab. IV), and SAk showed a monomorphism of this allele. In the females, in contrast, all strains had 2 alleles,

Table III

Genotype frequencies at 19 polymorphic protein loci in 8 strains of *Mnais* spp.; 2 other loci, Gpi-2 and Ldh-2, were monomorphic

Loci	Genotypes	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
Acp-2	67/67	0*(0.2)**	0 (0.1)	1 (0.3)	0 (0.1)	0 (0.6)	2 (1.8)	1 (0.5)	2 (0.5)
	67/60			0 (1.0)	1 (0.2)	4 (3.1)	7 (6.6)	1 (2.3)	1 (3.5)
	67/55	1 (1.8)	2 (0.6)	1 (1.4)	0 (0.4)	0 (0.5)	1 (1.8)	0 (0.8)	
	67/50	4 (2.3)	0 (1.1)		0 (0.5)	1 (1.3)			
	67/44	0 (0.4)		1 (1.0)	1 (0.8)	2 (1.1)		1 (0.3)	0 (0.4)
	67/35				0 (0.1)				
	60/60			2 (0.9)	0 (0.1)	6 (4.2)	6 (6.1)	2 (2.5)	7 (6.0)
	60/55			1 (2.5)	0 (0.5)	0 (1.3)	3 (3.3)	3 (1.5)	
	60/50				0 (0.8)	1 (3.5)			
	60/44			2 (1.8)	2 (1.1)	1 (3.1)		0 (0.5)	2 (1.4)
	60/35				0 (0.1)				
	55/55	5 (3.6)	1 (0.8)	2 (1.8)	0 (0.5)	2 (0.1)	1 (0.5)	0 (0.5)	
	55/50	6 (9.3)	1 (2.8)		0 (1.6)	0 (0.5)			
	55/44	3 (1.8)		4 (2.5)	6 (2.3)	0 (0.5)		0 (0.2)	
	55/35				0 (0.2)				
	50/50	8 (6.0)	4 (2.5)		2 (1.2)	0 (0.7)			
	50/44	0 (2.3)			4 (3.4)	5 (1.3)			
	50/35				1 (0.3)				
	44/44	1 (0.2)		0 (0.9)	0 (2.5)	0 (0.6)		0 (0.0)	0 (0.1)
	44/35				0 (0.4)				
	35/35				0 (0.0)				
	n	28	8	14	17	22[a]	20	8	12
Ak-1	36/36	5(1.9)	0 (0.1)	4 (3.0)	5 (2.8)		7 (5.5)	4 (2.8)	1 (1.0)
	36/33	3 (6.5)	2 (1.2)	3 (5.0)	5 (7.1)		7 (8.9)	2 (4.4)	0 (0.2)
	36/29	0 (2.7)	0 (0.6)		0 (2.3)		0 (1.1)		
	33/33	6 (5.5)	3 (3.4)	3 (2.0)	6 (4.5)	7 (9.8)	4 (3.6)	3 (1.8)	13 (12.1)
	33/29	7 (4.5)	3 (3.1)		2 (2.9)	16 (10.4)	2 (0.9)		
	29/29	1 (0.9)	1 (0.7)		2 (0.5)	0 (2.8)	0 (0.1)		
	n	22[a]	9	10	20[a]	23[a]	20	9	14
Ak-2	16/16					1 (4.3)	4 (3.2)	3 (3.4)	1 (2.6)
	16/14					0 (1.9)	8 (9.6)	4 (4.3)	10 (6.9)
	14/14	22	9	9	20	22 (21.1)	8 (7.2)	2 (1.4)	3 (4.6)
	n	22	9	9	20	23[a]	20	9	14
Aox-1	50/50					0 (0.0)			
	50/44					2 (0.4)			
	50/40					0 (1.4)			
	50/38					0 (0.1)			
	44/44				0 (0.0)	1 (0.7)		0 (0.1)	1 (0.9)
	44/40				2 (1.9)	3 (5.8)		2 (1.8)	5 (5.3)
	44/38					1 (0.4)			
	40/40	9 (6.4)	6 (6.1)	10	21 (21.1)	14 (11.5)	17	7 (7.1)	8 (7.9)
	40/38	4 (9.3)	2 (1.8)			1 (1.4)			
	38/38	6 (3.4)	0 (0.1)			0 (0.0)			
	n	19[a]	8	10	23	22	17	9	14
Aox-2	18/18			1 (0.2)					
	18/16			1 (2.6)					
	16/16	19	8	8 (7.2)	23	22	17	9	14
	n	19	8	10	23	22	17	9	14
G6pd-1	33/33	10(11.3)	4 (4.7)	1 (0.4)	3 (1.8)	0 (0.0)	2 (2.5)	1 (0.3)	1 (0.3)

Table III, continued

Loci	Genotypes	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
	33/30	10 (7.5)	5 (3.6)	2 (3.1)	6 (8.4)	2 (1.9)	10 (9.1)	1 (2.5)	2 (3.4)
	30/30	0 (1.3)	0 (0.7)	6 (5.4)	11 (9.8)	21 (21.1)	8 (8.5)	7 (6.2)	11 (10.3)
	n	20	9	9	20	23	20	9	14
G6pd-2	15/15	4 (5.5)	3 (4.0)	1 (0.7)	1 (2.5)	1 (1.1)	2 (4.3)	2 (1.0)	3 (2.2)
	15/13	13 (10.0)	6 (4.0)	3 (3.6)	12 (9.1)	8 (7.8)	14 (9.5)	2 (4.0)	5 (6.7)
	13/13	3 (4.5)	0 (1.0)	5 (4.7)	7 (8.5)	14 (14.1)	3 (5.3)	5 (4.0)	6 (5.2)
	n	20	9	9	20	23	19[a]	9	14
α-Gpdh-1	68/68	15	10	4 (4.2)	8 (8.0)	6 (9.1)	4 (4.2)		1 (0.2)
	68/65						2 (2.2)		0 (1.6)
	68/61			5 (4.7)	10 (9.9)	15 (11.5)			1 (1.1)
	65/65						0 (1.1)	4 (4.5)	3 (4.0)
	65/61							4 (3.0)	9 (5.4)
	61/61			1 (1.5)	3 (3.0)	1 (3.6)		0 (0.5)	0 (1.8)
	n	15	10	10	21	22[a]	6	8	14
α-Gpdh-2	54/54	8	5		6 (5.5)	9 (10.9)			
	54/51				11 (12.)	4 (2.8)			
	54/48					1 (0.7)			
	54/45					6 (4.2)			
	54/41					2 (1.4)			
	51/51			11	7 (6.5)	0 (0.2)	6	8	7 (7.9)
	51/48					0 (0.1)			
	51/45					0 (0.5)			7 (5.3)
	51/48					0 (0.1)			
	48/48					0 (0.0)			
	48/45					0 (0.1)			
	48/41					0 (0.0)			
	45/45					0 (0.4)			0 (0.9)
	45/41					0 (0.3)			
	41/41					0 (0.0)			
	n	8	5	11	24	22	6	8	14
α-Gpdh-3	15/15	3 (4.8)	4 (4.9)		7 (5.5)	4 (5.5)	0 (1.5)	1 (2.0)	3 (4.0)
	15/11	11 (7.4)	6 (4.2)		9 (12.0)	14 (11.0)	6 (3.0)	6 (4.0)	9 (7.0)
	11/11	1 (2.8)	0 (0.9)	11	8 (6.5)	4 (5.5)	0 (1.5)	1 (2.0)	2 (3.0)
	n	15	10	11	24	22	6[a]	8	14
Gpi-1	53/53					0 (0.0)			
	53/44					1 (0.2)			
	53/38					0 (0.6)			
	53/36					0 (0.2)			
	44/44	0 (0.0)		1 (0.6)	0 (0.2)	0 (1.0)			
	44/38	1 (0.4)		0 (2.3)	3 (2.0)	5 (5.2)			
	44/36	0 (0.6)		3 (1.6)	1 (1.6)	3 (1.6)			
	38/38	3 (3.7)	2 (3.0)	4 (2.3)	4 (5.0)	9 (6.6)	2 (4.3)	3 (2.8)	3 (4.0)
	38/36	11 (10.2)	7 (5.0)	2 (3.2)	9 (8.0)	0 (4.0)	14 (9.5)	4 (4.4)	9 (7.0)
	36/36	7 (7.1)	1 (2.0)	1 (1.1)	3 (3.2)	2 (0.6)	3 (5.3)	2 (1.8)	2 (3.0)
	n	22	10	11	20	20[a]	19[a]	9	14
Lap-2	72/72			0 (0.0)		0 (0.0)			
	72/70			0 (0.1)		0 (0.2)			
	72/68			0 (0.3)		1 (0.3)			
	72/64			0 (0.5)		0 (0.3)			
	72/59			1 (0.1)		0 (0.1)			
	72/56					0 (0.1)			

Table III, continued

Loci	Genotypes	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
	70/70	0 (0.1)	0 (0.6)	0 (0.1)	0 (0.3)	0 (0.9)	0 (0.3)		0 (0.0)
	70/68	1 (1.1)	0 (0.8)	0 (0.6)	0 (1.4)	2 (2.9)	3 (2.5)		1 (0.3)
	70/64	2 (1.9)	1 (1.8)	1 (0.9)	6 (2.8)	2 (2.9)	1 (0.3)		0 (0.4)
	70/59	1 (0.6)	2 (0.8)	1 (0.3)	0 (1.1)	2 (1.2)	0 (0.8)		0 (0.3)
	70/56	0 (0.1)	2 (0.5)		0 (0.1)	3 (0.6)			
	68/68	3 (2.9)	1 (0.2)	2 (1.4)	0 (1.6)	1 (2.2)	6 (6.3)	2 (1.5)	0 (0.9)
	68/64	5 (9.4)	0 (1.1)	5 (4.2)	10 (6.5)	9 (4.4)	0 (1.3)	2 (3.5)	2 (2.7)
	68/59	6 (3.1)	1 (0.5)	0 (1.2)	3 (5.1)	0 (1.9)	5 (3.8)	1 (0.4)	4 (2.2)
	68/56	2 (0.6)	0 (0.3)		1 (0.2)	0 (1.0)			
	64/64	13 (7.8)	3 (7.3)	3 (3.3)	2 (6.5)	1 (2.2)	0 (0.1)	3 (2.0)	2 (1.9)
	64/59	0 (5.2)	0 (1.1)	2 (1.9)	8 (5.1)	1 (1.9)	1 (0.2)	0 (0.5)	4 (3.1)
	64/56	0 (1.0)	0 (0.7)		0 (0.5)	0 (1.0)			
	59/59	2 (0.9)	0 (0.2)	0 (0.3)	0 (1.0)	0 (0.4)	0 (0.6)	0 (0.1)	0 (1.2)
	59/56	0 (0.3)	0 (0.2)		0 (0.2)	0 (0.2)			
	56/56	0 (0.0)	0 (0.1)		0 (0.0)	0 (0.1)			
	n	35[a]	10	15	30[a]	22	16	8	13
Lap-3	54/54	0 (0.1)		0 (0.2)	0 (0.8)	0 (0.0)	0 (0.1)	0 (0.3)	
	54/50	1 (1.2)		1 (1.5)	0 (1.5)	1 (0.4)	0 (0.2)	0 (0.5)	
	54/46	0 (1.4)		0 (0.1)	5 (3.3)	0 (0.2)	1 (0.6)	1 (1.0)	
	54/43	2 (0.6)		1 (1.5)	4 (2.3)	0 (0.4)	0 (0.5)	1 (0.3)	
	54/40	1 (0.5)		1 (0.4)	1 (1.2)		1 (0.5)	1 (0.7)	
	50/50	2 (3.2)	4 (3.1)	4 (3.5)	1 (0.7)	3 (2.9)	0 (0.2)	1 (0.3)	0 (0.2)
	50/46	6 (7.5)	1 (0.6)	0 (0.5)	1 (3.0)	2 (3.6)	0 (1.0)	0 (1.0)	1 (1.5)
	50/43	7 (3.3)	1 (3.1)	3 (3.0)	4 (2.1)	7 (6.2)	3 (0.7)	0 (0.3)	1 (0.8)
	50/40	3 (2.7)		2 (2.0)	2 (1.1)		0 (0.7)	1 (0.7)	1 (0.3)
	46/46	8 (4.5)	0 (0.0)	0 (0.0)	3 (3.3)	3 (1.1)	1 (1.4)	2 (1.0)	3 (3.3)
	46/43	1 (3.9)	0 (0.3)	1 (0.2)	4 (4.7)	2 (3.9)	1 (2.2)	0 (0.7)	6 (3.5)
	46/40	2 (3.2)		0 (1.5)	4 (2.3)		5 (2.2)	1 (1.3)	0 (1.5)
	43/43	0 (0.9)	2 (0.8)	0 (0.6)	1 (1.6)	4 (3.3)	1 (0.9)	0 (0.1)	0 (0.9)
	43/40	1 (1.4)		1 (0.9)	0 (1.6)		1 (1.8)	1 (0.4)	0 (0.8)
	40/40	1 (0.6)		0 (0.3)	0 (0.4)		0 (0.9)	0 (0.4)	1 (0.2)
	n	35	8	14	30	22	14	9	13
Lap-4	34/34			2 (1.8)	11 (11.1)	3 (2.7)			1 (0.9)
	34/30			2 (3.0)	2 (2.8)	2 (2.5)			0 (0.0)
	34/27			1 (0.5)	8 (6.9)	4 (5.4)			
	34/21					3 (1.8)			5 (4.6)
	30/30	20 (20.1)	3 (3.4)	2 (1.3)	1 (0.2)	1 (0.6)	0 (2.3)	0 (0.5)	1 (0.1)
	30/27		3 (2.3)	0 (0.4)	0 (0.9)	2 (2.5)			
	30/21	3 (2.8)				1 (0.8)	12 (7.5)	4 (3.0)	0 (1.3)
	27/27		0 (0.4)	0 (0.0)	1 (1.1)	4 (2.7)			
	27/21					1 (1.8)			
	21/21	0 (0.1)				0 (0.3)	4 (6.3)	4 (4.5)	6 (5.6)
	n	23	6	7	23	21	16[a]	8	13[b]
Ldh-1	40/40	0 (0.1)	1 (0.4)	0 (0.6)	2 (1.6)	1 (0.6)			
	40/38	2 (1.8)	2 (2.2)	5 (3.0)	7 (7.8)	4 (4.3)			
	40/36	1 (1.0)	0 (1.0)	0 (0.8)		0 (0.6)			
	38/38	9 (7.4)	4 (3.0)	3 (3.6)	10 (9.6)	9 (8.3)	5 (5.3)	5 (4.0)	3 (2.6)
	38/36	5 (8.3)	1 (2.2)	1 (1.8)		1 (2.2)	6 (5.3)	2 (4.0)	6 (6.9)
	36/36	4 (2.3)	2 (0.6)	1 (0.2)		1 (0.1)	1 (1.3)	2 (1.0)	5 (4.6)
	n	21	10	10	19	16	12	9	14
Pgm-1	36/36			0 (0.0)	0 (0.0)	0 (0.1)			

Table III, continued

Loci	Genotypes	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
	36/33			1 (0.6)	1 (0.4)	2 (1.2)			
	36/30			0 (0.3)	0 (0.5)	0 (1.2)			
	33/33	4(5.0)	5 (4.2)	5 (4.4)	6 (3.4)	7 (6.1)	1 (2.5)	6 (6.2)	4 (2.6)
	30/30	12 (10.0)	3 (4.6)	3 (4.4)	3 (8.8)	5 (12.1)	9 (6.0)	3 (2.5)	4 (6.9)
	30/30	4 (5.0)	2 (1.2)	2 (1.1)	9 (5.8)	8 (6.1)	2 (3.5)	0 (0.3)	6 (4.6)
	n	20	10	11	19*	22	12	9	14
Pgm-2	15/15	8 (6.6)	6 (6.4)	5 (4.4)	4 (5.0)	4 (4.1)	12	8	14
	15/12	7 (9.8)	4 (3.2)	4 (5.1)	12 (10.0)	10 (9.9)			
	12/12	5 (3.6)	0 (1.3)	2 (1.5)	4 (5.0)	6 (6.1)			
	n	20	10	11	20	20	12	8	14
Xdh-1	35/35	1 (1.3)		1 (0.2)	2 (3.2)	4 (2.8)	2 (3.5)	3 (2.8)	3 (2.6)
	35/31	7 (6.5)		1 (2.1)	10 (7.2)	8 (9.7)	12 (8.9)	4 (4.4)	6 (6.9)
	35/27	2 (1.9)		0 (0.2)	2 (2.4)	0 (0.7)			
	31/31	9 (7.9)	9	5 (4.9)	2 (4.1)	9 (8.5)	4 (5.5)	2 (1.8)	5 (4.6)
	31/27	2 (4.7)		3 (2.1)	4 (2.7)	2 (1.2)			
	27/27	2 (0.7)		0 (0.2)	0 (0.5)	0 (0.0)			
	n	23	9	10	20	23	18	9	14
Xdh-2	15/15	1 (2.3)	0 (0.4)		8 (6.6)	6 (6.3)			
	15/12	12 (9.3)	4 (3.1)		7 (9.8)	12 (11.5)			
	12/12	8 (9.3)	5 (5.4)	10	5 (3.6)	5 (5.3)	18	9	14
	n	21	9	10	20	23	18	9	14

* Observed number.

** Expected number, assuming random mating. Almost all of the observed distributions of genotypes agreed with the expected ones according to the Hardy-Weinberg equilibrium (χ^2-test, $p>0.05$).

a and b: Significantly different (χ^2-test, $p<0.05$ and $p<0.01$, respectively).

Xdh-1^{35} and *Xdh-1*^{31}, with nearly equal frequency, but they did not have *Xdh-1*^{27}. At the locus Xdh-2, the allele *Xdh-2*^{12} was dominant in the Kami-ishigama strains. This allele showed a monomorphism, and was very frequently obtained from, STk and the three female strains. In contrast, the allele *Xdh-2*^{15} was dominant in the Hisayama males.

GENETIC VARIABILITY WITHIN A STRAIN

The estimates of genetic variability within a strain are quantified by P (polymorphic loci) and $\bar{H}$ (average heterozygosity per individual), high values indicating high variability. In the males, high values of P, 0.857 and 0.810, and $\bar{H}$, 0.416 and 0.419 were obtained from the Hisayama strains (STh and ESh, respectively), whereas the lowest values of P (0.667 in SAk and STk) and $\bar{H}$ (0.307 in SAk) were found in the Kami-ishigama strains (Tab. V). In the females, the highest values of P (0.762) and $\bar{H}$ (0.353) were again found in the Hisayama strain (SIh). Thus, generally speaking, the genetic variability in the Hisayama strains was higher than that in the Kami-ishigama's strains in both sexes.

Table IV

Allele frequencies at 21 protein loci including monomorphic loci in 8 strains of *Mnais* spp.

Loci	Alleles	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
Acp-2	67	0.089	0.125	0.143	0.059	0.159	0.300	0.250	0.208
	60			0.250	0.088	0.439	0.550	0.500	0.708
	55	0.357	0.313	0.357	0.176	0.068	0.150	0.188	
	50	0.464	0.563		0.265	0.182			
	44	0.089		0.250	0.382	0.159		0.063	0.083
	35				0.029				
	*H**	0.641	0.570	0.727	0.740	0.719	0.585	0.648	0.448
Ak-1	36	0.295	0.111	0.550	0.375		0.525	0.556	0.071
	33	0.500	0.611	0.450	0.475	0.652	0.425	0.444	0.929
	29	0.205	0.278		0.150	0.348	0.050		
	H	0.621	0.537	0.495	0.611	0.454	0.541	0.494	0.133
Ak-2	16					0.043	0.400	0.556	0.429
	14	1.000	1.000	1.000	1.000	0.957	0.600	0.444	0.571
	H					0.083	0.480	0.494	0.490
Aox-1	50					0.045			
	44				0.043	0.182		0.111	0.250
	40	0.579	0.875	1.000	0.957	0.727	1.000	0.889	0.750
	38	0.421	0.125			0.045			
	H	0.488	0.219		0.083	0.434		0.198	0.375
Aox-2	18			0.150					
	16	1.000	1.000	0.850	1.000	1.000	1.000	1.000	1.000
	H			0.255					
G6pd-1	33	0.750	0.722	0.222	0.300	0.043	0.350	0.167	0.143
	30	0.250	0.278	0.778	0.700	0.957	0.650	0.833	0.857
	H	0.375	0.401	0.346	0.420	0.083	0.455	0.278	0.245
G6pd-2	15	0.525	0.667	0.278	0.350	0.217	0.474	0.333	0.393
	13	0.475	0.333	0.722	0.650	0.783	0.526	0.667	0.607
	H	0.499	0.444	0.401	0.455	0.340	0.499	0.444	0.477
α-Gpdh-1	68	1.000	1.000	0.650	0.619	0.614	0.833		0.107
	65						0.167	0.750	0.536
	61			0.350	0.381	0.386		0.250	0.357
	H			0.455	0.472	0.474	0.278	0.375	0.574
α-Gpdh-2	54	1.000	1.000		0.479	0.705			
	51			1.000	0.521	0.091	1.000	1.000	0.750
	48					0.023			
	45					0.136			0.250
	41					0.045			
	H				0.499	0.474			0.375
α-Gpdh-3	15	0.567	0.700		0.479	0.500	0.500	0.500	0.536
	11	0.433	0.300	1.000	0.521	0.500	0.500	0.500	0.464
	H	0.491	0.420		0.499	0.500	0.500	0.500	0.497
Gpi-1	53					0.025			
	44	0.023		0.227	0.100	0.225			
	38	0.409	0.550	0.455	0.500	0.575	0.474	0.556	0.536
	36	0.568	0.450	0.318	0.400	0.175	0.526	0.444	0.464

Table IV, continued

Loci	Alleles	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
	H	0.509	0.495	0.640	0.580	0.588	0.499	0.494	0.497
Gpi-2	14	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lap-2	72			0.033		0.023			
	70	0.057	0.250	0.067	0.100	0.205	0.125		0.038
	68	0.286	0.150	0.300	0.233	0.318	0.625	0.438	0.269
	64	0.471	0.350	0.467	0.467	0.318	0.063	0.500	0.385
	59	0.157	0.150	0.133	0.183	0.068	0.188	0.063	0.308
	56	0.029	0.100		0.017	0.068			
	H	0.667	0.760	0.669	0.684	0.746	0.555	0.555	0.683
Lap-3	54	0.057		0.107	0.167	0.023	0.071	0.167	
	50	0.300	0.625	0.500	0.150	0.364	0.107	0.167	0.115
	46	0.357	0.063	0.036	0.333	0.227	0.321	0.333	0.500
	43	0.157	0.313	0.214	0.233	0.386	0.250	0.111	0.269
	39	0.129		0.143	0.117		0.250	0.222	0.115
	H	0.738	0.508	0.671	0.771	0.666	0.755	0.772	0.651
Lap-4	34			0.500	0.696	0.357			0.269
	30	0.935	0.750	0.429	0.087	0.167	0.375	0.250	0.077
	27		0.250	0.071	0.217	0.357			
	23						0.625	0.750	0.654
	21	0.065				0.119			
	H	0.122	0.375	0.561	0.461	0.703	0.469	0.375	0.494
Ldh-1	40	0.071	0.200	0.250	0.289	0.188			
	38	0.595	0.550	0.600	0.711	0.719	0.667	0.667	0.429
	36	0.333	0.250	0.150		0.094	0.333	0.333	0.571
	H	0.529	0.595	0.555	0.411	0.439	0.444	0.444	0.490
Ldh-2	15	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1	36			0.045	0.026	0.045			
	33	0.500	0.650	0.636	0.421	0.477	0.458	0.833	0.429
	30	0.500	0.350	0.318	0.553	0.477	0.542	0.167	0.571
	H	0.500	0.455	0.492	0.517	0.542	0.497	0.278	0.490
Pgm-2	15	0.575	0.800	0.636	0.500	0.450	1.000	1.000	1.000
	12	0.425	0.200	0.364	0.500	0.550			
	H	0.489	0.320	0.463	0.500	0.495			
Xdh-1	35	239		0.150	0.400	0.348	0.444	0.556	0.429
	31	0.587	1.000	0.700	0.450	0.609	0.556	0.444	0.571
	27	0.174		0.150	0.150	0.043			
	H	0.568		0.465	0.615	0.507	0.494	0.494	0.490
Xdh-2	15	0.333	0.222		0.575	0.522			
	12	0.667	0.778	1.000	0.425	0.478	1.000	1.000	1.000
	H	0.444	0.346		0.489	0.499			

* Heterozygosity

Table V

Proportion of polymorphic loci (P) and average heterozygosity ($\bar{H}$) calculated for 8 strains of *Mnais* spp.

Sex	Populations	Proportion of polymorphic loci P (21 loci)	Average Heterozygocity $\bar{H}$ (21 loci)
Male	NAk	0.714	0.366
	SAk	0.667	0.307
	STk	0.667	0.343
	ESh	0.810	0.419
	STh	0.857	0.416
Female	NFk	0.667	0.336
	SIk	0.714	0.326
	SIh	0.762	0.353
Average		*0.732*	*0.358*

GENETIC DISTANCE BETWEEN STRAINS

The genetic diversity among the eight strains derived from the six forms in terms of Nei's genetic distance (D) and identity (I) was calculated from the allele frequency of each strain at the 21 respective protein loci (Tab. VI). In the males, the lowest value of D (0.0440) was obtained between NAk and SAk. Thus these two forms of *M. nawai* (f. *nawai* and f. *sahoi*) which inhabited the same locality (Kami-ishigama area), appear to be closely related male *M. pruinosa* (STk) was also collected in the Kami-ishigama area. However, it was further from NAk and SAk (D=0.2277 and 0.2263, respectively). The nearest strain to STh was the other male of *M. pruinosa* (ESh) (D=0.0588), which inhabited the same locality (Hisayama area) rather than a strain of the same form, STk from the Kami-ishigama area (D=0.1573).

In the females, low values of D, 0.0595, 0.0712 and 0.0856, were obtained between SIk and SIh, NFk and SIk, and NFk and SIh, respectively, suggesting that none of the genetic distances among the three female strains is significant regardless of species or habitat. The male strains farthest from all the females were NAk and SAk (both *M. nawai*), with D values ranging within 0.2248-0.3500. Lower values of D (0.1238-0.1818) were obtained between the female strains and STk among the male strains. It is suggested that these female strains are genetically closest to the male STh (*M. pruinosa*) from Kami-ishigama.

DISCUSSION

The genetic differentiation among the eight strains of *M. pruinosa* and *M. nawai* obtained from the localities of Kami-ishigama and Hisayama of Fukuoka in Japan, has been evaluated here on 21 protein loci. NEI (1975) stated that D values be-

Table VI
Values of Nei's genetic identity (*I*) and genetic distance (*D*) among the 8 strains of *Mnais* spp., based on 21 protein loci

D	*I*							
	NAk	SAk	STk	ESh	STh	NFk	SIk	SIh
NAk		0.9570	0.7964	0.8631	0.8541	0.7987	0.7076	0.7213
SAk	0.0440		0.7975	0.8416	0.8579	0.7958	0.7047	0.7282
STk	0.2277	0.2263		0.9022	0.8545	0.8836	0.8502	0.8338
ESh	0.1472	0.1725	0.1029		0.9429	0.8360	0.7945	0.8160
STh	0.1578	0.1533	0.1573	0.0588		0.8007	0.7650	0.8190
NFk	0.2248	0.2284	0.1238	0.1791	0.2223		0.9313	0.9179
SIk	0.3459	0.3500	0.1623	0.2301	0.2678	0.0712		0.9422
SIh	0.3268	0.3172	0.1818	0.2033	0.1997	0.0856	0.0595	

tween subspecies range within 0.004-0.351 (usually 0.1-0.2). THORPE (1982) concluded that, if the *D* value was more than 0.16 between two strains, they could not be regarded as the same species, even if they were identified as the same by morphological and physiological characteristics and by the level of reproductive isolation. However, he then indicated that a *D* range of 0.02-3.9 (usually 0.22-1.6) appears to indicate interspecific variation within a genus for a large number of organisms, except birds.

Figure 1 is a dendrogram by UPGMA clustering based on the data of genetic distance (*D*). All the males examined in this study could be delineated into two intraspecific groups based on the above two criteria (NEI, 1975; THORPE, 1982). The groups are NAk and SAk (*M. nawai*) as group I, ESh, STh and STk (*M. pruinosa*) as group II. All *D* values obtained within each group were less than 0.16, indicating the level of conspecific variation. The genetic distance between these two groups (*D*=0.1923, data not shown) was outside the range of intraspecific variation defined by THORPE (1982). Thus these results for the males suggest that f. *nawai* and f. *sahoi* of *M. nawai* can be categorized as a single species, but that f. *esakii* and f. *strigata* of *M. pruinosa* should be regarded as a valid species separate from *M. nawai*. This result agrees with the morphometric and geographical classification by SUZUKI (1981, 1984, 1986) who gave species status to both *M. nawai* and *M.*

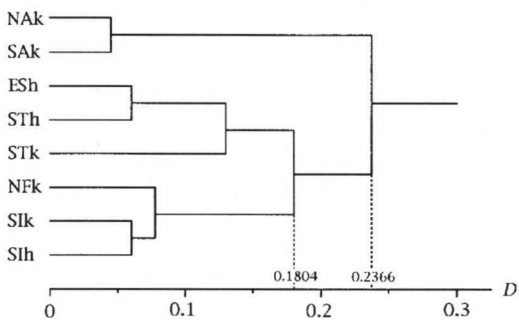

Fig. 1. Dendrogram of the eight strains derived from six forms of *Mnais* spp. based on UPGMA clustering of the data in Table VI.

pruinosa.

In the females, the *D* values obtained between them were low, ranging from 0.0595-0.0856, indicating that they are genetically related and within the intraspecific range, though they include two different species, *M. nawai* and *M. pruinosa*. They were the closest to STk (f. *strigata* of *M. pruinosa*) inhabiting the Kami-ishigama area, but the *D* value to group II was calculated as 0.1804, indicating a level of interspecific variation. Two loci, Pgm-2 and Xdh-2, both showed monomorphism in the females, but considerable polymorphism in the males (Tab. III). The possibility that these loci are linked to sex chromosomes should be investigated in more detail.

KNOPF (1977) determined the genetic status of many odonates. Almost all of the genetic distances between species showed interspecific variation according to the criteria of NEI (1975) and THORPE (1982), with *D* values being greater than 0.16. Among six species of the subgenus *Gomphus*, *G. exilis, G. hodgesi, G. lividus, G. australis, G. diminutus* and *G. minutus*, however, genetic distances were less than 0.16 in *D* value, which indicates a conspecific variation, though every species status is accepted. KNOPF (1977) analyzed genetic distances using starch gel electrophoresis, which was different from our electrophoretic conditions using polyacrylamide gel. In generally, protein bands are more clearly detected on polyacrylamide gel than on starch gel. His study could thus have estimated lower variations than this one.

The range of *P* value quantifying the genetic variability within a strain for different groups of organisms is very large, but usually within 0.2-0.4 (average 0.3) (NEI, 1975). As shown in Table V, *P* values ranged from 0.667-0.857 (average 0.732), meaning that 14 to 18 of the 21 proteins examined showed polymorphism. Each *P* value is much larger than the usual indicated by NEI (1975). $\bar{H}$ values (average 0.358) were also larger than the average of 0.1 (NEI, 1975). In the dragonfly genus *Gompus*, *H* values ranging from 0-0.0852 (grand mean 0.0221) were obtained among 23 species based on 22 genetic loci using starch gel electrophoresis (KNOPF, 1977). Knopff also reported that nine species in the Libellulidae had a mean $\bar{H}$ of 0.049, the highest value of all species examined being 0.1111 obtained from *Ladona deplanata*. As KNOPF (1977) noted, these *H* values are much lower than those obtained for most other insects that have been investigated. Both his electrophoretic conditions and the dragonfly species examined were different from ours, so that to estimate the genetic variability.

KOJIMA et al. (1970) described that some enzymes, such as carboxylesterase, phosphatase and amylase, had high polymorphism in *Drosophila melanogaster* and *D. simulans*. The level of genetic variability in protein loci for non glucose--metabolizing enzymes (e.g., carboxylesterase, alcohol dehydrogenase, octanol dehydrogenase and xanthin dehydrogenase) generally seems to be much higher than that of glucose-metabolizing enzymes (e.g., hexokinase, aldolase, isocitorate dehydrogenase, α-glycerolphosphate dehydrogenase, glucose-6-phosphate dehydro-

genase and phosphoglucomutase) (LEWONTIN, 1974). Of the 10 proteins examined in our study, all showed polymorphism in some strains. Though only one of these, xanthine dehydrogenase, was mentioned by LEWONTIN (1974), our isozyme analysis could have been carried out using the data from these higher polymorphic proteins. To estimate the genetic variability, further examinations using multiple numbers and kinds of proteins are needed in the future.

If the rate of molecular evolution has been constant among *Mnais* spp. of Japan, the divergence time (t years) between the two strains can be estimated from the genetic distance (D) between them using the following equation (NEI, 1975) :

$$t = 5 \times 10^6 D$$

The divergence time of f. *nawai* and f. *sahoi* of *M. nawai* is calculated as approximately 220,000 years. Similarly, these two forms diverged from two other males, f. *esakii* and f. *strigata* of *M. pruinosa*, more than 900,000 years ago. However, this estimation from electrophoretic data is subject to a large amount of error, because the genetic distances could be profoundly influenced by the sample size of loci, migration between strains and a bottleneck effect (CHAKRABORTY & NEI, 1977) in either strain.

CONCLUSION

The results obtained from the present study suggest the following conclusions.

(1) For the male strains, two polymorphic forms of *M. pruinosa*, f. *esakii* and f. *strigata*, and also two forms of *M. nawai*, f. *nawai* and f. *sahoi*, belong to single species, respectively.
(2) The males of *M. pruinosa* and *M. nawai* belong to separate species.
(3) All the females are genetically close to each other, though they include those of two different species, *M. pruinosa* and *M. nawai*.

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