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Inheritance of Mitochondrial DNAs and Allozymes in the Female Hybrid Lineage of Two Japanese Pond Frog Species

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ABSTRACT—In order to elucidate the nucleotide sequence divergence of mitochondrial DNAs between two Japanese pond frog species *Rana nigromaculata* and *R. brevipoda*, and the mode of inheritance of cytoplasmic genomes in the female hybrid lineages of the two species, the cleavage patterns of mtDNAs digested with 10 restriction endonucleases were examined by agarose gel electrophoresis using a total of 52 frogs including *Rana nigromaculata* and *R. brevipoda*, their reciprocal hybrids and the backcross offspring (B_1 and B_2) derived from female hybrids by crossing with the paternal species. The cleavage patterns for mtDNA of *Rana nigromaculata* were different from those of *R. brevipoda* digested with all the restriction endonucleases used except *Eco*RV. The nucleotide sequence divergence of mtDNAs between these two species was roughly estimated to be 8.5%. The cleavage patterns for mtDNAs of the reciprocal hybrids and the B_1 and B_2 offspring were clearly similar to those of the maternal species, and paternal mtDNAs could not be detected. On the other hand, the proportions of original maternal nuclear genes at the 22 allozyme loci were 50% in the reciprocal hybrids, 21.9% or 25.6% in the B_1 offspring, and 7.5% in the B_2 offspring. These results demonstrate that nuclear genomes decrease the original maternal constitution in the female hybrid lineages generationally, whereas the mtDNAs are inherited maternally during repeated backcrossing.

INTRODUCTION

The two Japanese pond frog species, *Rana nigromaculata* and *R. brevipoda* (= *R. porosa brevipoda*), are nearly completely isolated by male hybrid sterility (Moriya, 1951, 1960; Kawamura, 1962; Kawamura and Nishioka, 1977). By contrast, the females of reciprocal hybrids between these two species are fertile to a large extent. Kawamura and Nishioka (1978) examined the change of reproductive capacity in the descendants of their reciprocal hybrids, and clarified that female hybrids were more or less inferior to their parents in reproductive capacity. There was scarcely any improvement in females of the B_1, B_2 or B_3 generation on an average in reproductive capacity as compared with their female parents. In each of four lineages derived from the reciprocal female hybrids by backcrossing with the paternal and maternal species, males were distinctly inferior to their sisters in reproductive capacity.

Mitochondrial DNA (mtDNA) has been shown to be predominantly maternally inherited in *Xenopus* (Dawid and Blackler, 1972), *Drosophila* (Reilly and Thomas, 1980) and *Heliothis* (Lansman *et al.*, 1983). Maternal inheritance of mtDNA has also been observed in horse-donkey hybrids (Hutchison *et al.*, 1974), the rat *Rattus norvegicus* (Buzzo *et al.*, 1978; Francisco *et al.*, 1979; Hayashi *et al.*, 1978; Kroon *et al.*, 1978), the white-footed mouse *Peromyscus polionotus* (Avise *et al.*, 1979), *Mus* (Gyllensten *et al.*, 1985), human (Giles *et al.*, 1980), *Onchorhynchus* (Ginatulina and Maksimovich, 1994), chicken-quail hybrids (Watanabe *et al.*, 1985) and ducks (Lin *et al.*, 1990). Strictly maternal inheritance of mtDNA has been demonstrated during extensive backcrossing in both directions between *Mus domesticus* and *Mus spretus* (Gyllensten *et al.*, 1985) and between *Heliothis virescens* and *H. subflexa* (Lansman *et al.*, 1983).

As to the nuclear genomes, the preferential expression of a maternal or paternal allele has been reported frequently in the somatic cells of hybrids (Castro-Sierra and Ohno, 1968; Hitzeroth *et al.*, 1968; Whitt *et al.*, 1972, 1973; Honjo and Reeder, 1973; Yamaguchi and Goldberg, 1974; Schmidtke *et al.*, 1976; Etkin, 1977; Durica and Krider, 1977), but rarely in the germ cells of hybrids (Brown and Blackler, 1972; Vogel and Chen, 1976; Graf *et al.*, 1977) (Elinson, 1981).

In the present study, the cleavage patterns of mtDNAs were examined using two Japanese pond frog species *Rana nigromaculata* and *R. brevipoda*, their reciprocal hybrids and the B_1 and B_2 offspring derived from fertile female hybrids backcrossed with the paternal species, in order to elucidate the nucleotide sequence divergence of mtDNAs between two species and the mode of inheritance of cytoplasmic genomes

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during repeated backcrossing of Japanese pond frogs. The enzymes and blood proteins encoded by nuclear genes were also examined to confirm the expression of parental genes and the chromosomal genome constitutions in the reciprocal hybrids and the backcross offspring.

MATERIALS AND METHODS

A total of 52 mature frogs was used in the present study (Table 1). These included eight *Rana nigromaculata*, eight *R. brevipoda* (= *R. p. brevipoda*), 24 reciprocal hybrids, and 12 B_1 and B_2 offspring derived from reciprocal female hybrids. All the crossing experiments were carried out by artificial fertilization method during the breeding seasons of 1990~1994. Ovulation was accelerated by injection of suspension of bullfrog pituitaries into the body cavity. Tadpoles were fed on boiled spinach. Metamorphosed frogs were fed on two-spotted crickets. The 14 enzymes extracted from livers and skeletal muscles and the three blood proteins of the above 52 frogs were analyzed by means of starch gel electrophoresis according to the method of Nishioka *et al.* (1980, 1992) with a slight modification, whereas the amylase of blood sera was examined by polyacrylamide gel electrophoresis (Table 2). Each enzyme was detected by the agar-overlay method outlined by Harris and Hopkinson (1976). The detection of blood proteins was made with the amido-black staining method.

Mitochondria were isolated from livers or ovaries. Tissues were homogenized in a decuple volume of STE buffer cooled with ice (0.25 M sucrose, 0.03 M Tris-HCl, 0.01 M EDTA, 0.11 M NaCl, pH 7.6), and centrifuged at 800 × g for 10 min at 2°C. The supernatant was centrifuged at 10,000 × g for 10 min at 2°C. MtDNAs were purified by CsCl ethidium bromide density gradient centrifugation described by Yonekawa *et al.* (1980) as follows.

Mitochondria obtained from each frog were lysed completely by suspending the pellet in 3.6 ml (final volume) of 0.6% sarcosyl, 10 mM EDTA and 10 mM Tris-HCl (pH 8.0). The lysate was then dialyzed against the above buffer for 4 hr at room temperature. The volume of lysate was adjusted to 3.6 ml by the same buffer, and 3.6 grams of solid CsCl and 0.24 ml of 4.6 mg/ml ethidium bromide were added to the lysate and mixed well. Then the lysate was centrifuged at 36,000 rpm (Hitachi RPS-40 rotor) for 40 hr at 20°C. The fraction containing

Table 1. Number of specimens used in the present study

Kind[a]	Crossing	Abbreviation[b]	No. of frogs		
			Female	Male	Total
Rana nigromaculata	(N) NN ♀ × (N) NN ♂	(N) NN	4	4	8
Rana brevipoda	(B) BB ♀ × (B) BB ♂	(B) BB	4	4	8
F_1 hybrid	(N) NN ♀ × (B) BB ♂	(N) NB	8	5	13
F_1 hybrid	(B) BB ♀ × (N) NN ♂	(B) BN	8	3	11
B_1 offspring	(N) NB ♀ × (B) BB ♂	(N) NB × BB	4	0	4
B_1 offspring	(B) BN ♀ × (N) NN ♂	(B) BN × NN	4	0	4
B_2 offspring	{(N) NB × BB} ♀ × (B) BB ♂	(N) NB × BB × BB	1	3	4
	Total		33	19	52

[a] F_1 represents the first filial generation of interspecific hybrid. The first and second generation backcrosses are abbreviated to B_1 and B_2, respectively.
[b] N and B refer to a genome of *Rana nigromaculata* and a genome of *R. brevipoda*, respectively. The letters in parentheses indicate sources of cytoplasm.

Table 2. Enzymes and blood proteins analyzed in the present study

Enzyme or blood protein	Abbreviation	E.C.No.	Sample	Buffer system[a]
Adenosine deaminase	ADA	3.5.4.4	Skeletal muscle	T-C pH 7.0
Alcohol dehydrogenase	ADH	1.1.1.1	Liver	T-B-E pH 8.0
Aldolase	ALD	4.1.2.13	Skeletal muscle	T-C pH 7.0
Amylase	AMY	3.2.1.1	Blood serum	T-G pH 8.6 (PAGE)
Enolase	ENO	4.2.1.11	Skeletal muscle	T-C pH 7.0
Glycerol-3-phosphate dehydrogenase	GPD	1.1.1.8	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	5.3.1.9	Skeletal muscle	T-B-E pH 8.0
Hexokinase	HK	2.7.1.1	Liver	T-C pH 7.0
Isocitrate dehydrogenase	IDH	1.1.1.42	Skeletal muscle	T-C pH 7.0
Lactate dehydrogenase	LDH	1.1.1.27	Skeletal muscle	T-C pH 6.0
Malate dehydrogenase	MDH	1.1.1.37	Skeletal muscle	T-C pH 6.0
Malic enzyme	ME	1.1.1.40	Skeletal muscle	T-C pH 7.0
Peptidase	PEP	3.4.11	Liver	T-B-E pH 8.0
Superoxide dismutase	SOD	1.15.1.1	Skeletal muscle	T-C pH 7.0
Sorbitol dehydrogenase	SORDH	1.1.1.14	Liver	T-C pH 7.0
Serum albumin	Alb	–	Blood serum	T-B-E pH 8.0
Hemoglobin	Hb	–	Erythrocyte	T-B-E pH 8.6
Serum protein C	Prot-C	–	Blood serum	T-B-E pH 8.0

[a] T-C, T-B-E and T-G represent Tris-citrate buffer, Tris-borate-EDTA buffer and Tris-glycine buffer, respectively. Amylase was analyzed by polyacrylamide gel electrophoresis.

closed circular mtDNA was collected, extracted three times with CsCl-saturated isopropanol to remove the dye and dialyzed against 0.1 mM EDTA (pH 8.0) for 20 hr with two changes of the medium. The mtDNA solutions were stored at -20°C until used.

Ten kinds of six-base recognizing restriction endonucleases, *Bam*HI, *Eco*RI, *Eco*RV, *Hae*II, *Hin*dIII, *Hpa*I, *Pst*I, *Pvu*II, *Sac*I and *Xba*I, were purchased from TaKaRa. The mtDNAs were digested by incubating at 37°C for 2 hr with appropriate amounts of the enzymes under conditions described by the suppliers. Agarose slab gel (1% Sigma agarose) electrophoresis was carried out in the standard TAE

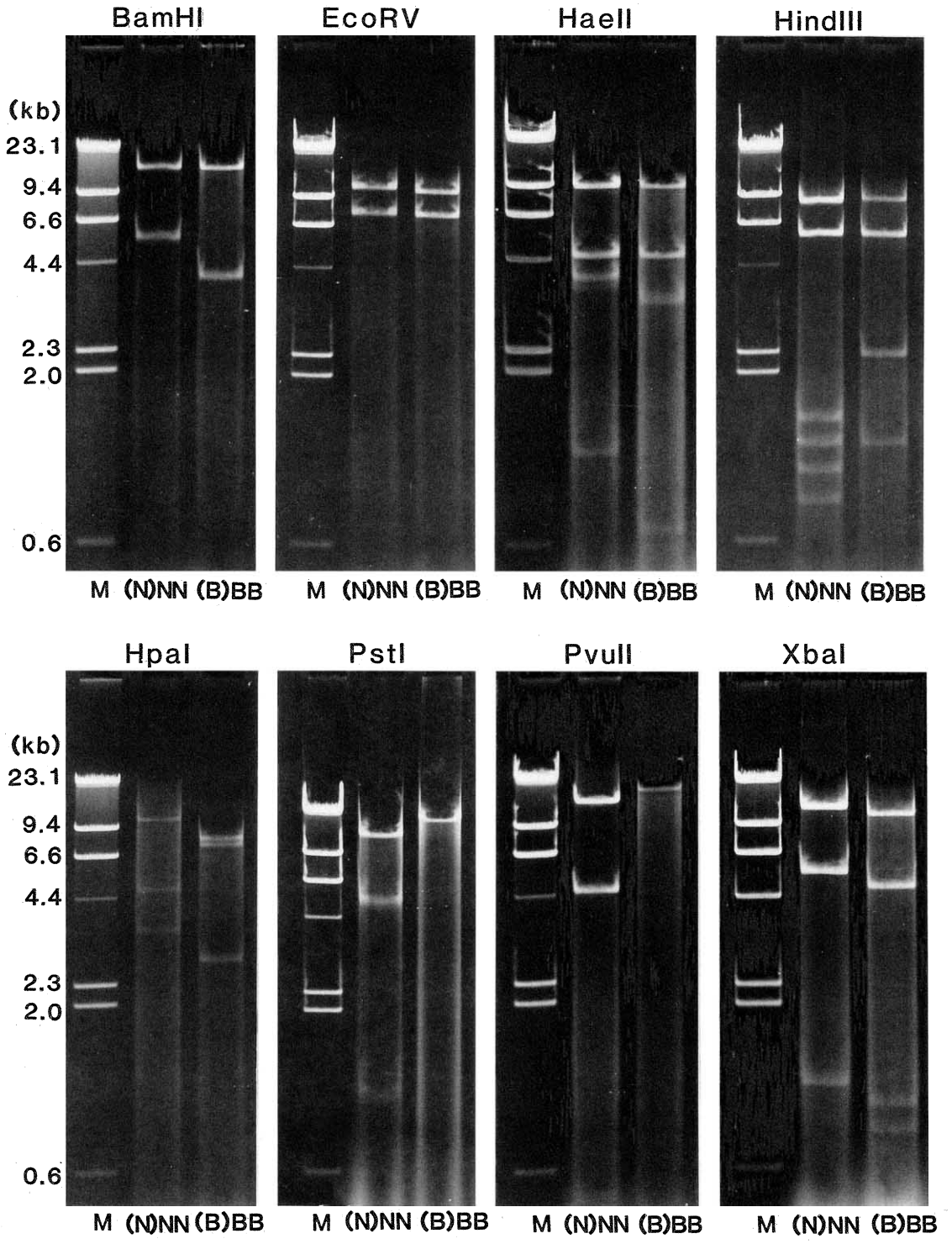

Fig. 1. The cleavage patterns for mtDNAs of *Rana nigromaculata* and *R. brevipoda* digested with eight restriction endonucleases. M = λ-DNA digested with *Hin*dIII as molecular weight standards. The cleavage patterns for mtDNAs of *R. nigromaculata* were different from those of *R. brevipoda* digested with all the enzymes used except *Eco*RV.

buffer (0.04 M Tris, 0.001 M EDTA, 0.02 M sodium acetate, pH 8.3). After electrophoresis, the gels were stained with 0.1 μg/ml ethidium bromide and photographed under ultraviolet light. λ-DNA digested with *Hin*dIII was used as molecular weight standards. Nucleotide sequence divergence was calculated by the method of Gotoh *et al.* (1979).

The abbreviations of N and B refer to a genome of *Rana nigromaculata* and a genome of *R. brevipoda*, respectively. The letters in parentheses indicate sources of cytoplasm. The first and second generation backcrosses are abbreviated to B_1 and B_2, respectively.

RESULTS

Mitochondrial DNAs

Figure 1 shows a comparison of the cleavage patterns for mtDNAs of two Japanese pond frog species, *Rana nigromaculata* and *R. brevipoda*, digested with eight restriction endonucleases, *Bam*HI, *Eco*RV, *Hae*II, *Hin*dIII, *Hpa*I, *Pst*I, *Pvu*II and *Xba*I. The cleavage patterns for mtDNA of *R. nigromaculata* were different from those of *R. brevipoda* digested with all the enzymes used except *Eco*RV. The molecular weight of each restriction fragment of these mtDNAs was estimated by comparing relative mobility to those of molecular weight standards (Table 3). The molecular weight of total mtDNA genome was estimated to be 19.1 ± 0.19 kb in *Rana nigromaculata* and 18.4 ± 0.18 kb in *R. brevipoda.* The numbers of common and different cleavage sites between *R. nigromaculata* and *R. brevipoda* were inferred from those of the fragments. The nucleotide sequence divergence between these two species was roughly estimated to be 8.5%.

Figures 2 and 3 show the cleavage patterns for mtDNAs of the reciprocal hybrids between these two species and the B_1 and B_2 offspring digested with three restriction endonucleases, *Bam*HI, *Hin*dIII and *Pst*I. The cleavage patterns for mtDNAs of 13 hybrids (N)NB between female *Rana nigromaculata* and male *R. brevipoda*, four B_1 offspring (N)NB × BB obtained from matings between female hybrids and male *R. brevipoda*, and four B_2 offspring (N)NB × BB × BB obtained from matings between female B_1 offspring and male *R. brevipoda* were clearly similar to those of *R. nigromaculata*. Likewise the cleavage patterns for mtDNAs of 11 hybrids (B)BN between female *R. brevipoda* and male *R. nigromaculata*, and four B_1 offspring (B)BN × NN obtained from matings between female hybrids and male *R. nigromaculata* were clearly similar to those of *R. brevipoda*. The mtDNA cleavage patterns observed in the hybrids and the B_1 and B_2 offspring are summarized in Table 4. The paternal mtDNA cleavage patterns could not be detected at all in these hybrids nor backcrosses. These results demonstrate that mtDNAs were primarily maternally inherited in these hybrids and backcrosses.

Nuclear genome constitutions

Figures 4 and 5 show the electrophoretic patterns of enzymes and blood proteins in *R. nigromaculata*, *R. brevipoda*, their reciprocal hybrids and the B_1 and B_2 offspring. The electrophoretic patterns of two species were clearly different from each other at all the 22 diagnostic loci (Table 5). They were *NN* and *BB* in genotype, respectively. The electrophoretic patterns of each hybrid consisted of the sum of those of the two parental species at all the 22 loci. They were *NB* or *BN* in genotype. In the four B_1 offspring (N)NB × BB, 7~10 of 20 loci showed hybrid pattern, *NB* in genotype, and the remaining 10~13 loci revealed the *brevipoda* pattern, *BB* in genotype (Table 5). In the four B_1 offspring (B)BN × NN, 7~14 of 22 loci showed hybrid pattern, *BN* in genotype, and the other 8~15 loci revealed the *nigromaculata* pattern, *NN* in genotype (Table 5). Of the four B_2 offspring (N)NB × BB × BB, one showed the *brevipoda* pattern, *BB* in genotype, at all the 20 loci examined. The remaining three showed the hybrid pattern, *NB* in

Table 3. Restriction fragments of mtDNAs from *Rana nigromaculata* and *R. brevipoda*

Enzyme	Site	(N) NN			(B) BB			No. of sites	
		No.[a]	Size (kb)	Total[b]	No.[a]	Size (kb)	Total[b]	c_i[c]	d_i[d]
*Bam*HI	GGATCC	2	13.6 5.3	18.9	2	14.6 3.9	18.5	1	2
*Eco*RI	GAATTC	3	8.8 5.6 4.4	18.8	1	18.6	18.6	1	2
*Eco*RV	GATATC	2	11.1 7.5	18.6	2	11.1 7.5	18.6	2	0
*Hae*II	PuGCGCPy	4	8.7 4.4 3.7 1.1	17.9	5	8.7 4.4 3.2 0.7 0.6	17.6	3	3
*Hin*dIII	AAGCTT	6	8.6 5.8 1.4 1.2 1.0 0.8	18.8	4	8.6 5.8 2.3 1.2	17.9	4	2
*Hpa*I	GTTAAC	3	11.1 4.6 3.4	19.1	3	8.4 7.6 2.8	18.8	1	4
*Pst*I	CTGCAG	3	13.4 5.2 1.0	19.6	1	18.7	18.7	1	2
*Pvu*II	CAGCTG	2	15.4 4.5	19.9	1	19.3	19.3	1	1
*Sac*I	GAGCTC	2	13.6 5.9	19.5	1	18.7	18.7	1	1
*Xba*I	TCTAGA	3	13.4 5.5 1.0	19.9	4	11.2 4.8 0.9 0.7	17.6	1	5
Average (Sequence divergence)		3.0		19.1	2.4		18.4	1.6 (8.5%)	2.2

[a] Number of restriction fragments.
[b] Total length (kb) of fragments.
[c] Number of common cleavage sites between *R. nigromaculata* and *R. brevipoda*.
[d] Number of different cleavage sites between *R. nigromaculata* and *R. brevipoda*.

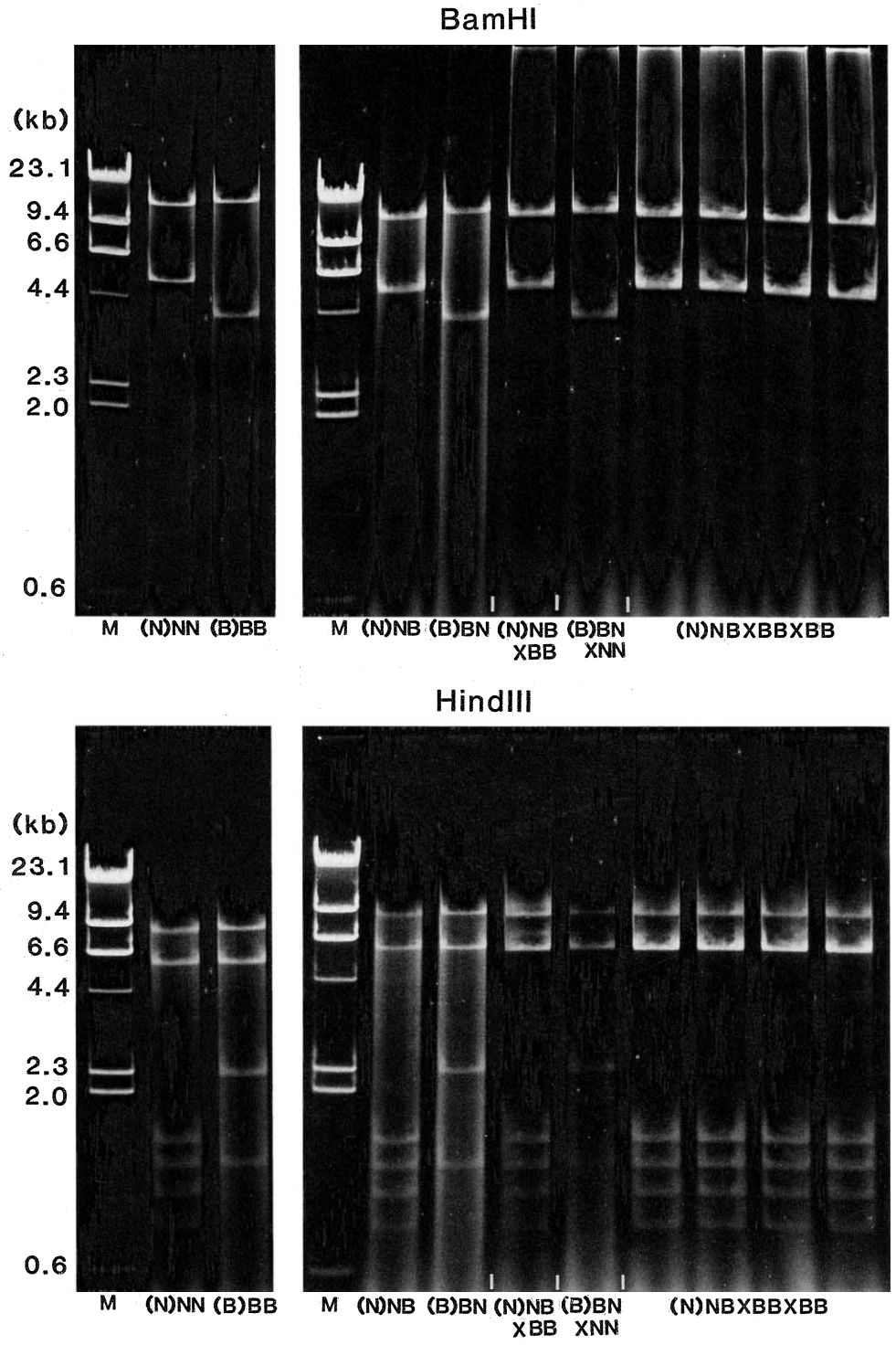

Fig. 2. The cleavage patterns for mtDNAs of the reciprocal hybrids between two species and the B_1 and B_2 offspring digested with *Bam*HI and *Hin*dIII. M = λ-DNA digested with *Hin*dIII as molecular weight standards. The cleavage patterns for mtDNAs of the reciprocal hybrids and the B_1 and B_2 offspring were clearly similar to those of the maternal parent.

genotype, at 3~5 loci and the *brevipoda* pattern, *BB* in genotype, at the other 15~17 loci (Table 5).

The proportions of original maternal genes were 50% in the reciprocal hybrids, 21.9% or 25.6% in the B_1 offspring, and 7.5% in the B_2 offspring (Table 5). Thus it was found that the nuclear genomes decreased the original maternal constitution in the female hybrid lineages generationally.

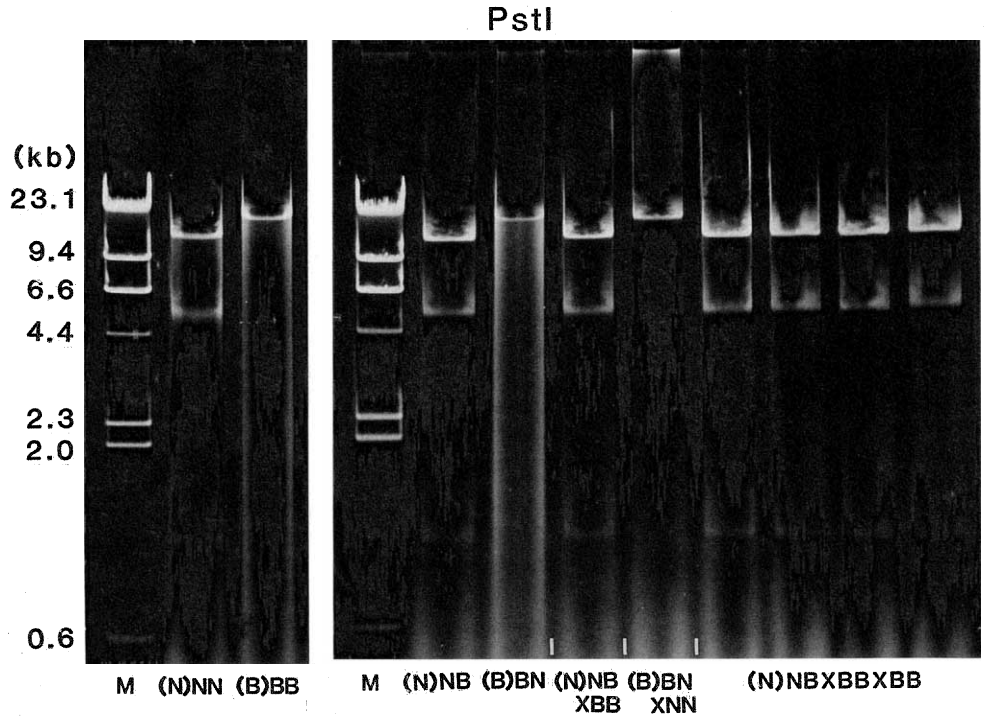

Fig. 3. The cleavage patterns for mtDNAs of the reciprocal hybrids between two species and the B_1 and B_2 offspring digested with *Pst*I. M = λ-DNA digested with *Hin*dIII as molecular weight standards. The cleavage patterns for mtDNAs of the reciprocal hybrids and the B_1 and B_2 offspring were clearly similar to those of the maternal parent.

Table 4. MtDNA cleavage patterns of the reciprocal hybrids and the backcross offspring

Kind	(N) NB	(B) BN	(N) NB × BB				(B) BN × NN				(N) NB × BB × BB			
Ind.no.	1~13	1~11	1	2	3	4	1	2	3	4	1	2	3	4
*Bam*HI	N	B	N	N	N	N	B	B	B	B	N	N	N	N
*Hin*dIII	N	B	N	N	N	N	B	B	B	B	N	N	N	N
*Pst*I	N	B	N	N	N	N	B	B	B	B	N	N	N	N

DISCUSSION

The present results demonstrate that the inheritance of mtDNAs is not governed by the same rules that apply to chromosome genes. As shown in Fig. 6, mtDNAs are maternally inherited; the mtDNAs of the original maternal parent are transmitted in the fertile female hybrid lineage during extensive backcrossing. On the other hand, both maternal and paternal nuclear genes are expressed in the reciprocal hybrids, and chromosomal genomes decrease the constitution of the original maternal parent in the fertile female hybrid lineage generationally (Fig. 6). Nishioka and Ohtani (1986) examined the lampbrush chromosome constitution of oocytes in 100 female backcrosses produced from female hybrids between female *brevipoda* and male *nigromaculata* by backcrossing with male *nigromaculata*. Of 1300 bivalents in total, 656 were BN in chromosome constitution and the other 644 were NN in chromosome constitution. These results showed that 656 (25.2%) of all the lampbrush chromosomes were derived from maternal species of the hybrids. Theoretically, in the course of extensive backcrossing of the fertile female hybrids with paternal species, the original maternal chromosomes or genes decrease to 50% in reciprocal hybrids, to 25.0% in the B_1 offspring and to 12.5% in the B_2 offspring. In the present study, the proportions of original maternal genes were 50.0% in reciprocal hybrids, 21.9% or 25.6% in the B_1 offspring and 7.5% in the B_2 offspring. These values almost coincide with the expected values stated above (χ^2 = 0~2.29, $P > 0.13$).

Nishioka, Ohtani and Sumida (1980, 1987), Nishioka and Ohtani (1986), Nishioka and Sumida (1994a, b), Sumida and Nishioka (1994a, b) and Sumida (1996) reported several linkage groups of enzyme and blood protein loci, color mutant genes and sex-linked genes in the *Rana nigromaculata* group and the Japanese brown frog *Rana japonica*. According to these studies, 10 linkage groups were established in the *Rana nigromaculata* group; *Alb*, *ADH-2* and albino gene *b* on

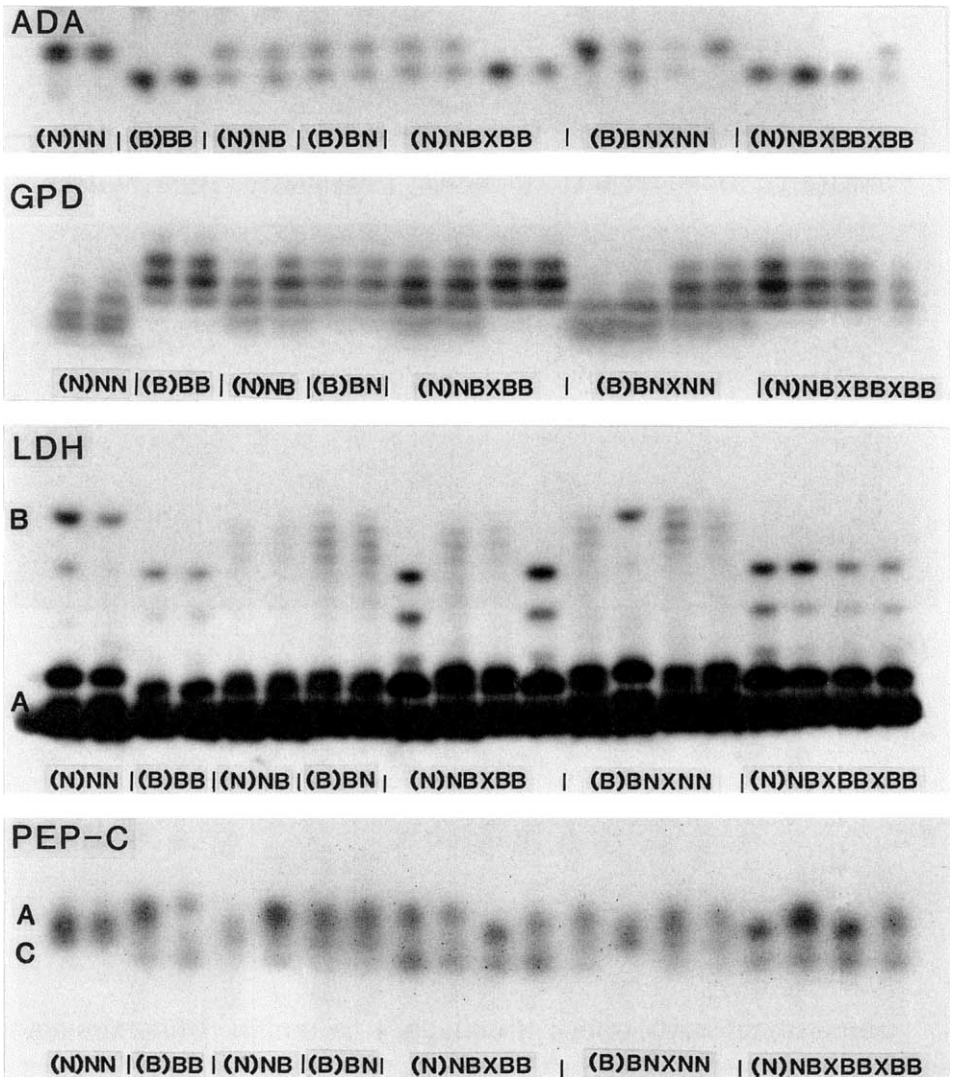

Fig. 4. The electrophoretic patterns of ADA, GPD, LDH and PEP-C in *R. nigromaculata*, *R. brevipoda*, the reciprocal hybrids and the B_1 and B_2 offspring. In the PEP-C patterns, the substrate L-leucyl-alanine also detected the PEP-A.

chromosome No. 1; *GPD*, *SOD-1*, *PEP-C*, *ME-2* and albino genes *a* and *c* on chromosome No. 2; *MDH-1*, *ME-1*, albino gene *e* and olive mutant gene on chromosome No. 3; *LDH-B*, *PEP-B*, *HK*, *MPI*, *SORDH* and *ENO* on chromosome No. 4; *PEP-A* on chromosome No. 5; *Hb* and *IDH-1* on chromosome No. 6; blue mutant gene on chromosome No. 8; *Prot-C*, *ALD* and albino gene *d* on chromosome No. 9; *PEP-D*, *EST-1*, *EST-2*, *EST-4* and *EST-5* on chromosome No. 10; *ADA* on chromosome No. 11. The present results gave definite evidence of linkages between the *SOD-1* and *PEP-C* loci, between *LDH-B* and *HK* loci, between *SORDH* and *ENO* loci and between *Hb* and *IDH-1* loci (Table 5), where the recombination rates were 16.7% ($\chi^2 = 5.33$, $P < 0.03$), 8.3% ($\chi^2 = 8.33$, $P < 0.004$), 8.3% and 16.7%, respectively.

The evidence presented here demonstrates that the mtDNAs were primarily maternally inherited during repeated backcrossing. The earliest evidence for maternal inheritance of mtDNA in animals came from the crossing experiments performed by Dawid and Blackler (1972) on two species of *Xenopus*. The studies of maternal inheritance using intra- and interspecific or intergeneric hybrids and repeated backcrosses also produced results concordant with the present ones (Avise *et al.*, 1979; Buzzo *et al.*, 1978; Francisco *et al.*, 1979; Giles *et al.*, 1980; Ginatulina and Maksimovich, 1994; Gyllensten *et al.*, 1985; Hayashi *et al.*, 1978; Kroon *et al.*, 1978; Lansman *et al.*, 1983; Lin *et al.*, 1990; Reilly and Thomas, 1980; Watanabe *et al.*, 1985).

According to Hutchison *et al.* (1974), two mechanisms for maternal inheritance are considered: (1) paternal mitochondria may be incapable of replication during development of the fertilized eggs, (2) maternal inheritance may result from a quantitative preponderance of maternal mitochondria in the zygotes. Gillham (1978) has raised the possibility that maternal inheritance in a single generation cross could be determined by interaction between nuclear and mitochondrial genes. It is believed that as little as 5% paternal

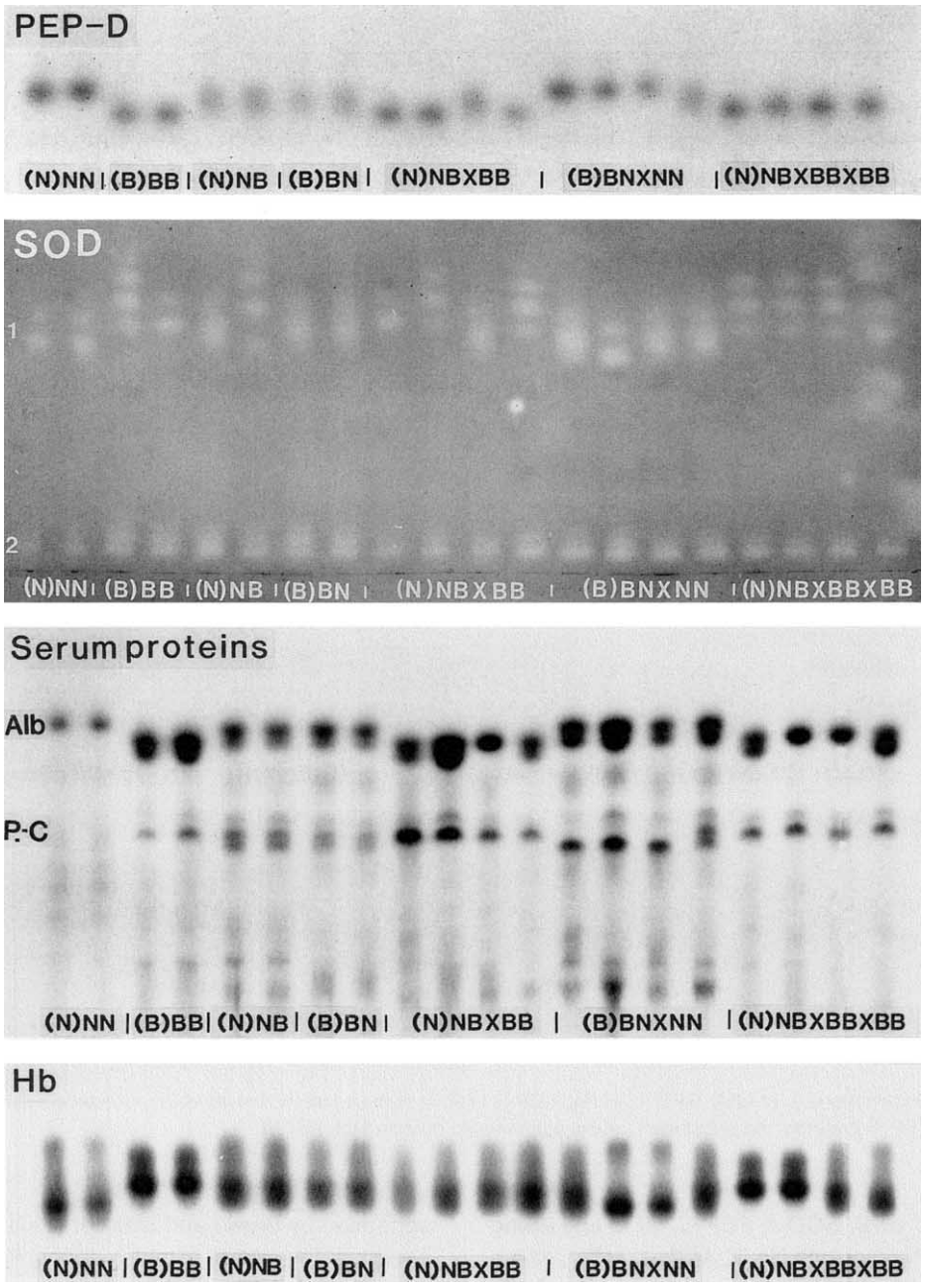

Fig. 5. The electrophoretic patterns of PEP-D, SOD, serum proteins and Hb in *R. nigromaculata*, *R. brevipoda*, the reciprocal hybrids and the B_1 and B_2 offspring.

mtDNAs will have been detectable in this experiment, however the presence of smaller amounts of paternal mtDNAs cannot be excluded. In *Xenopus laevis*, an egg contains more than 10^8 mtDNA molecules, whereas each sperm is estimated to contain about 100 molecules (Dawid, 1966; Dawid and Blackler, 1972). 10^{-6} of the mtDNA paternally derived would be below the limit of detection in this experiment. Nevertheless, some papers have shown paternal leakage of mtDNAs in the interspecific backcrosses of *Drosophila* (Kondo *et al.*, 1990) or *Mus* (Gyllensten *et al.*, 1991) using sufficiently sensitive techniques such as the Southern hybridization or the polymerase chain reaction. Kaneda *et al.* (1995) demonstrated that in intraspecific hybrids of *Mus musculus*, the paternal mtDNA genome got into the egg but was rapidly destroyed, whereas in interspecific hybrids between *Mus musculus* and *M. spretus*, the paternal mtDNA contribution persisted at least up until the neonate stage of development. It would be of interest to know whether leaky paternal inheritance of mtDNAs occurs during early development in the female hybrid lineages of the two Japanese pond frog species. These female hybrid lineages are considered to be very useful and attractive systems for examining the paternal mtDNA contribution during

Table 5. Genotypes at 22 loci of enzymes and blood proteins in the reciprocal hybrids and the backcross offspring

Kind	(N) NB	(B) BN	(N) NB × BB				(B) BN × NN				(N) NB × BB × BB			
Ind.no.	1~13	1~11	1	2	3	4	1	2	3	4	1	2	3	4
ADA	*NB*	*BN*	*NB*	*NB*	*BB*	*BB*	*NN*	*BN*	*BN*	*NN*	*BB*	*BB*	*BB*	*NB*
ADH-2	*NB*	*BN*	*BB*	*BB*	*BB*	*NB*	*NN*	*BN*	*NN*	*NN*	*BB*	*BB*	*BB*	*BB*
ALD	*NB*	*BN*	*NB*	*BB*	*NB*	*NB*	*NN*	*NN*	*NN*	*BN*	*NB*	*BB*	*BB*	*BB*
AMY	*NB*	*BN*	*BB*	*BB*	*NB*	*NB*	*NN*	*BN*	*BN*	*BN*	*BB*	*BB*	*BB*	*BB*
ENO	*NB*	*BN*	*BB*	*NB*	*BB*	*NB*	*BN*	*BN*	*NN*	*NN*	*BB*	*BB*	*BB*	*BB*
GPD	*NB*	*BN*	*NB*	*NB*	*BB*	*BB*	*NN*	*NN*	*BN*	*BN*	*BB*	*BB*	*BB*	*NB*
GPI	*NB*	*BN*	*BB*	*BB*	*BB*	*NB*	*BN*	*BN*	*BN*	*NN*	*BB*	*BB*	*BB*	*BB*
HK	*NB*	*BN*	*BB*	*NB*	*NB*	*BB*	*BN*	*NN*	*BN*	*NN*	*BB*	*BB*	*BB*	*BB*
IDH-1	*NB*	*BN*	*NB*	*NB*	*BB*	*NB*	*BN*	*NN*	*NN*	*NN*	*BB*	*BB*	*NB*	*NB*
LDH-B	*NB*	*BN*	*BB*	*NB*	*NB*	*BB*	*BN*	*NN*	*BN*	*BN*	*BB*	*BB*	*BB*	*BB*
MDH-1	*NB*	*BN*	*NB*	*BB*	*NB*	*BB*	*NN*	*NN*	*NN*	*BN*	*NB*	*BB*	*NB*	*BB*
ME-1	*NB*	*BN*	*NB*	*BB*	*NB*	*BB*	*NN*	*NN*	*NN*	*BN*	*NB*	*BB*	*NB*	*BB*
ME-2	*NB*	*BN*	*BB*	*NB*	*NB*	*BB*	*NN*	*NN*	*NN*	*BN*	*BB*	*BB*	*BB*	*NB*
PEP-A	*NB*	*BN*	–	–	–	–	*BN*	*NN*	*BN*	*BN*	–	–	–	–
PEP-B	*NB*	*BN*	–	–	–	–	*BN*	*NN*	*BN*	*NN*	–	–	–	–
PEP-C	*NB*	*BN*	*BB*	*NB*	*BB*	*BB*	*BN*	*NN*	*BN*	*BN*	*BB*	*BB*	*BB*	*BB*
PEP-D	*NB*	*BN*	*BB*	*BB*	*NB*	*BB*	*NN*	*NN*	*NN*	*BN*	*BB*	*BB*	*BB*	*BB*
SOD-1	*NB*	*BN*	*BB*	*BB*	*NB*	*BB*	*BN*	*NN*	*BN*	*BN*	*BB*	*BB*	*BB*	*BB*
SORDH	*NB*	*BN*	*BB*	*NB*	*BB*	*NB*	*BN*	*BN*	*BN*	*NN*	*BB*	*BB*	*BB*	*BB*
Alb	*NB*	*BN*	*BB*	*BB*	*BB*	*BB*	*BN*	*BN*	*BN*	*BN*	*BB*	*BB*	*BB*	*BB*
Hb	*NB*	*BN*	*NB*	*NB*	*NB*	*NB*	*BN*	*NN*	*NN*	*BN*	*BB*	*BB*	*NB*	*NB*
Prot-C	*NB*	*BN*	*BB*	*BB*	*BB*	*BB*	*NN*	*NN*	*NN*	*BN*	*BB*	*BB*	*BB*	*BB*
N(%)[a]	50	50	21.9				74.4				7.5			
B(%)[b]	50	50	78.1				25.6				92.5			

[a] Average percentage of genes derived from *R. nigromaculata*.
[b] Average percentage of genes derived from *R. brevipoda*.

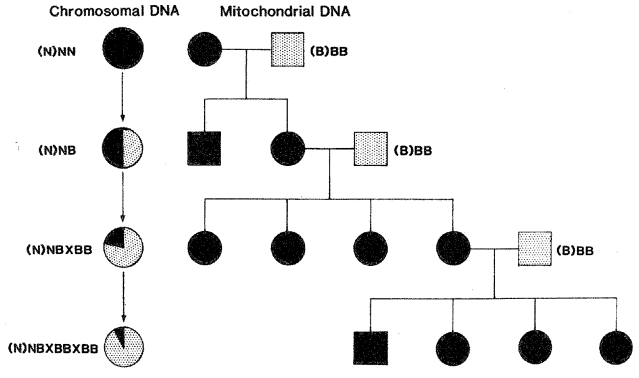

Fig. 6. Scheme illustrating the inheritance of the nuclear and cytoplasmic genomes in the female hybrid lineage of two Japanese pond frog species. The chromosomal genomes decrease the original maternal constitution generationally, whereas the mitochondrial genomes are inherited maternally during repeated backcrossing. Circular, female; square, male; black, *nigromaculata* genome; dot, *brevipoda* genome.

early development. Subsequent examination using polymerase chain reaction will clarify this point.

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REFERENCES

Avise JC, Lansman RA, Shade RO (1979) The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. Genetics 92: 279–295

Brown DD, Blackler AW (1972) Gene amplification proceeds by a chromosome copy mechanism. J Mol Biol 63: 75–83

Buzzo K, Fouts DL, Wolstenholme DR (1978) EcoRI cleavage site variations of mitochondrial DNA molecules from rats. Proc Natl Acad Sci USA 75: 909–913

Castro-Sierra E, Ohno S (1968) Allelic inhibition at the autosomally inherited gene locus for liver ADH in chicken-quail hybrids. Biochem Genet 1: 323–338

Dawid IB (1966) Evidence for the mitochondrial origin of frog egg cytoplasmic DNA. Proc Natl Acad Sci USA 56: 269–276

Dawid IB, Blackler AW (1972) Maternal and cytoplasmic inheritance of mitochondrial DNA in *Xenopus*. Develop Biol 29: 152–161

Durica DS, Krider HM (1977) Studies on the ribosomal cistrons in interspecific *Drosophila* hybrids. I. Nucleolar dominance. Develop Biol 59: 62–74

Elinson RP (1981) Genetic analysis of developmental arrest in an amphibian hybrid (*Rana catesbeiana*, *Rana clamitans*). Develop Biol 81: 167–176

Etkin LD (1977) Preferential expression of the maternal allele for alcohol dehydrogenase (ADH) in the amphibian hybrid *Ambystoma mexicanum* (axolotl) × *Ambystoma texanum*. Develop Biol 60: 93–100

Francisco JF, Brown GG, Simpson MV (1979) Further studies on types

A and B rat mtDNAs: cleavage maps and evidence for cytoplasmic inheritance in mammals. Plasmid 2: 426–436
Giles RE, Blanc H, Cann HM, Wallace DC (1980) Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci USA 77: 6715–6719
Gillham NW (1978) Organelle Heredity. Raven Press, New York
Ginatulina LK, Maksimovich AA (1994) Inheritance of mitochondrial DNA in artificial hybrids of pink (*Oncorhynchus gorbuscha* Walb.) and masu (*Oncorhynchus masou* Brev.) salmon. Genetika 30: 1248–1250
Gotoh O, Hayashi J-I, Yonekawa H, Tagashira Y (1979) An improved method for estimating sequence divergence between related DNAs from changes in restriction endonuclease cleavage sites. J Mol Evol 14: 301–310
Graf JD, Karch F, Moreillon MC (1977) Biochemical variation in the *Rana esculenta* complex: a new hybrid form related to *Rana perezi* and *Rana ridibunda*. Experientia 33: 1582–1584
Gyllensten U, Wharton D, Josefsson A, Wilson AC (1991) Paternal inheritance of mitochondrial DNA in mice. Nature 352: 255–257
Gyllensten U, Wharton D, Wilson AC (1985) Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. J Hered 76: 321–324
Harris H, Hopkinson DA (1976) Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publ Co, Amsterdam
Hayashi J-I, Yonekawa H, Gotoh O, Watanabe J, Tagashira Y (1978) Strictly maternal inheritance of rat mitochondrial DNA. Biochem Biophys Res Commun 83: 1032–1038
Hitzeroth H, Klose J, Ohno S, Wolf U (1968) Asynchronous activation of parental alleles at the tissue specific gene loci observed in hybrid trout during early development. Biochem Genet 1: 287–300
Honjo T, Reeder R (1973) Preferential transcription of *Xenopus laevis* ribosomal RNA in interspecies hybrids between *Xenopus laevis* and *Xenopus mulleri*. J Mol Biol 80: 217–228
Hutchison CA, Newbold JE, Potter SS, Edgell MH (1974) Maternal inheritance of mammalian mitochondrial DNA. Nature 251: 536–538
Kaneda H, Hayashi J-I, Takahama S, Taya C, Lindahl KF, Yonekawa H (1995) Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. Proc Natl Acad Sci USA 92: 4542–4546
Kawamura T (1962) On the names of some Japanese frogs. J Sci Hiroshima Univ Ser B Div 1, 20: 181–193
Kawamura T, Nishioka M (1977) Aspects of the reproductive biology of Japanese anurans. In "The Reproductive Biology of Amphibians" Ed by DH Taylor and SI Guttman, Plenum Press, New York and London, pp 103–139
Kawamura T, Nishioka M (1978) Descendants of reciprocal hybrids between two Japanese pond-frog species, *Rana nigromaculata* and *Rana brevipoda*. Sci Rep Lab Amphibian Biol Hiroshima Univ 3: 399–419
Kondo R, Satta Y, Matsuura ET, Ishiwa H, Takahata N, Chigusa SI (1990) Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. Genetics 126: 657–663
Kroon AM, de Vos WM, Bakker H (1978) The heterogeneity of rat-liver mitochondrial DNA. Biochim Biophys Acta 519: 269–273
Lansman RA, Avise JC, Huettel MD (1983) Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. Proc Natl Acad Sci USA 80: 1969–1971
Lin L-Y, Cheng I-P, Tzeng CS, Huang PC (1990) Maternal transmission of mitochondrial DNA in Ducks. Biochem Biophys Res Commun 168: 188–193
Moriya K (1951) On isolating mechanisms between the two subspecies of the pond frogs, *Rana nigromaculata*. J Sci Hiroshima Univ Ser B Div 1, 12: 47–56
Moriya K (1960) Studies on the five races of the Japanese pond frog, *Rana nigromaculata* Hallowell. III. Sterility in interracial hybrids. J Sci Hiroshima Univ Ser B Div 1, 18: 125–156
Nishioka M, Ohtani H (1986) Detection of chromosomes bearing the loci for blue and olive mutations in *Rana nigromaculata*. Sci Rep Lab Amphibian Biol Hiroshima Univ 8: 1–27
Nishioka M, Ohtani H, Sumida M (1980) Detection of chromosomes bearing the loci for seven kinds of proteins in Japanese pond frogs. Sci Rep Lab Amphibian Biol Hiroshima Univ 4: 127–184
Nishioka M, Ohtani H, Sumida M (1987) Chromosomes and the sites of five albino gene loci in the *Rana nigromaculata* group. Sci Rep Lab Amphibian Biol Hiroshima Univ 9: 1–52
Nishioka M, Sumida M (1994a) The position of sex-determining genes in the chromosomes of *Rana nigromaculata* and *Rana brevipoda*. Sci Rep Lab Amphibian Biol Hiroshima Univ 13: 51–97
Nishioka M, Sumida M (1994b) The differences in recombination rate between the male and female in *Rana nigromaculata* and *Rana brevipoda*. Sci Rep Lab Amphibian Biol Hiroshima Univ 13: 99–136
Nishioka M, Sumida M, Ohtani H (1992) Differentiation of 70 populations in the *Rana nigromaculata* group by the method of electrophoretic analyses. Sci Rep Lab Amphibian Biol Hiroshima Univ 11: 1–70
Reilly JG, Thomas CA (1980) Length polymorphisms, restriction site variation, and maternal inheritance of mitochondrial DNA of *Drosophila melanogaster*. Plasmid 3: 109–115
Schmidtke J, Kuhl P, Engel W (1976) Transitory hemizygosity of paternally derived alleles in hybrid trout embryos. Nature 260: 319–320
Sumida M (1996) Inheritance and linkage analysis of ten enzyme and blood protein loci in the Japanese brown frog *Rana japonica*. Biochem Genet 34: 375–388
Sumida M, Nishioka M (1994a) Geographic variability of sex-linked loci in the Japanese brown frog *Rana japonica*. Sci Rep Lab Amphibian Biol Hiroshima Univ 13: 173–195
Sumida M, Nishioka M (1994b) A pronounced sex difference when two linked loci of the Japanese brown frog *Rana japonica* are recombined. Biochem Genet 32: 361–369
Vogel P, Chen PS (1976) Genetic control of LDH isozymes in the *Rana esculenta* complex. Experientia 32: 304–307
Watanabe T, Mizutani M, Wakana S, Tomita T (1985) Demonstration of the maternal inheritance of avian mitochondrial DNA in chicken-quail hybrids. J Exp Zool 236: 245–247
Whitt GS, Childers WF, Cho PL (1973) Allelic expression at enzyme loci in an intertribal hybrid sunfish. J Hered 64: 55–61
Whitt GS, Cho PL, Childers WF (1972) Preferential inhibition of allelic isozyme synthesis in an interspecific sunfish hybrid. J Exp Zool 179: 271–282
Yamaguchi T, Goldberg E (1974) Asynchronous expression of glucose-6-phosphate dehydrogenase in splake trout embryos. Develop Biol 39: 63–68
Yonekawa H, Moriwaki K, Gotoh O, Watanabe J, Hayashi J-I, Miyashita N, Petras ML, Tagashira Y (1980) Relationship between laboratory mice and the subspecies *Mus musculus domesticus* based on restriction endonuclease cleavage patterns of mitochondrial DNA. Jpn J Genet 55: 289–296

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