Sex Differences in Polymorphism and Expression of AAT-1 in the Hiroshima Population of Buergeria buergeri (Anura, Rhacophoridae)

Authors: Atsumi, Shigeru, Ohta, Shigeru, and Sumida, Masayuki

Source: Zoological Science, 15(4) : 469-475

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/0289-0003(1998)15[469:SDIPAE]2.0.CO;2

ZOOLOGICAL SCIENCE **15**: 469–475 (1998)

Sex Differences in Polymorphism and Expression of AAT-1 in the Hiroshima Population of *Buergeria buergeri* (Anura, Rhacophoridae)

Shigeru Atsumi[1], Shigeru Ohta[2] and Masayuki Sumida[1]*

[1]*Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Higashihiroshima 739-8526, Japan*
[2]*High School attached to Hiroshima-Denki Institute of Technology, Hiroshima 736-0022, Japan*

ABSTRACT—*Buergeria buergeri* is female heterozygous in sex determination; chromosome pair No. 7 in this species is a pair of sex chromosomes of the ZZ/ZW type. Genetic analysis of AAT-1 variants was carried out to elucidate the mode of inheritance of this locus by starch-gel electrophoresis using field-caught females and males and their offspring produced by artificial crossings. The results showed that the *AAT-1* locus is sex-linked and that alleles are expressed on the Z chromosome, but not the W chromosome. It is evident that the AAT-1 gene of female offspring is hemizygous and that the allele present is on the Z chromosome, which is derived from the male parent.

INTRODUCTION

Sex-determining mechanisms can be demonstrated by (1) cytological observation of sex chromosomes, (2) sex-reversal and breeding experiments, (3) segregation analyses of sex-linked genes, and (4) H-Y antigen typing. Sex-determining mechanisms have been assigned in more than 50 species of amphibians by these methods (reviewed by Hillis and Green, 1990; Schmid *et al*., 1991; Solari, 1994).

Cytogenetic studies have determined the sex-determining mechanisms in the following species: XX/XY type in *Triturus alpestris*, *T. vulgaris* and *T. helveticus* (Schmid *et al*., 1979), *Rana esculenta* (Schempp and Schmid, 1981), *Gastrotheca riobambae* (Schmid *et al*., 1983), *T. cristatus* and *T. marmoratus* (Sims *et al*., 1984), *Hydromantes italicus*, *H. ambrosii*, *H. imperalis*, *H. flavus* and *H.* sp. *nova* (Nardi *et al*., 1986), *G. ovifera* (Schmid *et al*., 1988), *Centrolenella antisthenesi* (Schmid *et al*., 1989) and *G. pseustes* (Schmid *et al*., 1990), ZZ/ZW type in *Leiopelma hamiltoni* (Stephenson *et al*., 1972), *Pyxicephalus adspersus* (Schmid, 1980), and *Xenopus laevis* (Schmid and Steinlein, 1991), and XX/XY and ZZ/ZW types in *Rana rugosa* (Nishioka *et al*., 1994). The sex-determining mechanism was demonstrated by analyzing the sex ratio of the progeny of sex-reversed females in *Ambystoma* (Humphrey, 1942, 1945, 1957), *Bufo bufo* (Ponse, 1942), *Bufo viridis* (Ueda, 1990), *Xenopus* (Gallien, 1953; Chang and Witschi, 1955, 1956), and several *Rana* species (Kawamura and Nishioka, 1977). Sex-linked loci were also detected in *Rana clamitans* and *R. catesbeiana* (Elinson, 1981, 1983), the *Rana pipiens* complex (Wright and Richards, 1983; Wright *et al*., 1983), *Pleurodeles waltl* (Ferrier *et al*., 1983), *P. poireti* (Dournon *et al*., 1984), *Xenopus laevis* (Graf, 1989), the *Rana nigromaculata* group (Nishioka and Sumida, 1994) and *Rana japonica* (Sumida and Nishioka, 1994). The sex-determining mechanism was deduced in *Pelodytes punctatus* and four other species by the titer of the H-Y antigen found more abundantly in the heterogametic sex (Wachtel *et al*., 1975; Engel and Schmid, 1981).

The Japanese bell-ring frog, *Buergeria buergeri*, is an endemic species in which the sex-determining mechanism was confirmed both by cytological observation and by sex-reversal and breeding experiments (Ohta, 1986, 1987; Schmid *et al*., 1993). The sex chromosomes are of the ZZ/ZW type (female heteromorphic). The Z chromosome has a satellite and a nucleolar organizer at the tip of its long arm, whereas the W chromosome has none. Allozyme analysis of genetic divergence among geographic populations of this species revealed that the allelic frequencies at the *AAT-1* locus differed between females and males of several populations (Atsumi *et al*., unpublished).

In the present study, genetic analysis of AAT-1 variants was carried out to elucidate the mode of inheritance of this locus using field-caught male and female frogs of the Hiroshima population and their offspring produced by artificial crossings.

* Corresponding author: Tel. +81-824-24-7482;
FAX. +81-824-24-0739.

MATERIALS AND METHODS

A total of 101 adult frogs (51 females and 50 males) of the Hiroshima population (from Ugakyo, Asa-cho, Hiroshima city) of *Buergeria buergeri* were used in the present study. Twenty males were collected in July, 1996, the other 30 males and 51 females in June, 1997. Skeletal muscle of each individual was used for starch-gel electrophoretic analysis by the method of Nishioka *et al.* (1980), and aspartate aminotransferase (AAT) was detected by the agar-overlay method outlined by Harris and Hopkinson (1976). Crossing experiments were performed by artificial insemination using two females homozygous and two males heterozygous at the *AAT-1* locus. The embryos and tadpoles were reared in a moist chamber at a constant temperature of 20~22°C; the tadpoles were fed on boiled spinach. The tadpole stages follow those of Taylor and Kollros' (1946) table. The tail-tips of tadpoles at TK stage X~XIII were fixed in Navashin's fluid and stained with Heidenhain's iron hematoxylin following Ohta's (1986) method. After the sex of each tadpole was determined by counting the number of nucleoli in the nucleus of individual cells (Fig. 1), the AAT-1 phenotypes were analyzed by starch-gel electrophoresis using the tail-tip muscles.

RESULTS

The electrophoretic analysis of AAT-1 of 101 field-caught female and male *B. buergeri* revealed six phenotypes controlled by four alleles (Table 1, Fig. 2). The electrophoretic bands corresponding to single alleles at the *AAT-1* locus were named A, B, C and D in the order of mobility from fast to slow; the encoding alleles for bands A, B, C and D were named *a*, *b*, *c* and *d*, respectively. Phenotypes with two bands were given composite names; AB and AC were observed. Of 50 males, 35 were B in phenotype, two were C in phenotype, 12 were BC in phenotype, and one was AB in phenotype. On the other hand, of 51 females, 36 were B in phenotype, 13 were C in phenotype, one was A in phenotype, and one was D in phenotype. None of the 51 females showed phenotypes with two bands. As to the allelic frequencies, allele *b* was the highest in frequency, followed by allele *c* in both females and males. The two other alleles were either found only rarely or not at all. There were no significant differences between the actual

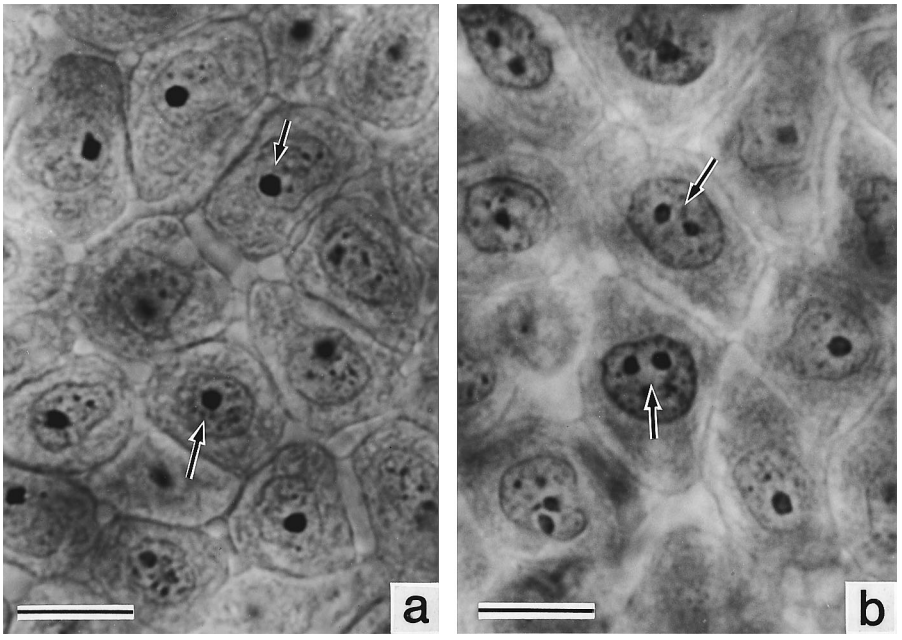

Fig. 1. Photomicrographs of tail-tip epidermal cells of tadpoles. (**a**) Tadpole having single nucleolus per nucleus (1-nu). (**b**) Tadpole having two nucleoli per nucleus (2-nu). Scale bars equal 10 μm. The arrows point to the nucleoli.

Table 1. Phenotypes and allelic frequencies at the *AAT-1* locus in the Hiroshima population of *B. buergeri*

Sex	No. of frogs	Phenotypes (Expected numbers)						Allelic frequencies†			
		A	B	C	D	AB	BC	*a*	*b*	*c*	*d*
Female	51	1 (0.0) (1.0*)	36 (25.4) (36.0*)	13 (3.3) (13.0*)	1 (0.0) (1.0*)	0 (1.4) (0.0*)	0 (18.4) (0.0*)	0.02	0.71	0.25	0.02
Male	50	0 (0.0)	35 (34.4)	2 (1.3)	0 (0.0)	1 (0.8)	12 (13.3)	0.01	0.83	0.16	0.00

* Values calculated from allelic frequencies based on the hypothesis that *AAT-1* is sex-linked.
† Allelic frequencies were rounded off to two decimal.

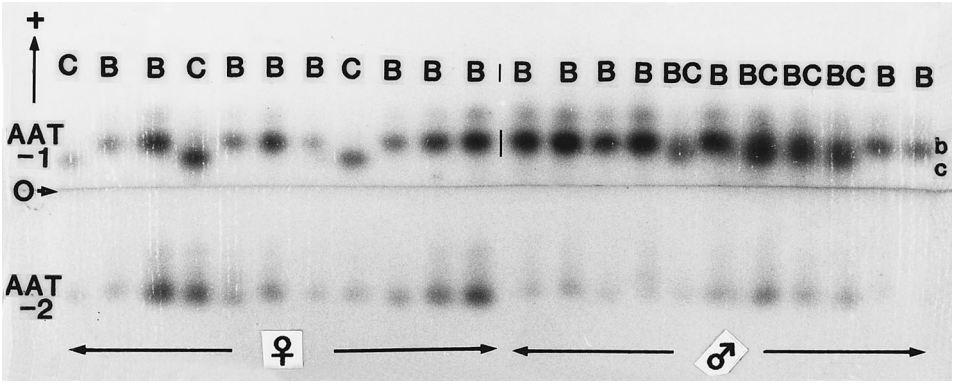

Fig. 2. Electrophoretic patterns of aspartate aminotransferase (AAT) in 11 females and 11 males from the Hiroshima population of *Buergeria buergeri*. The two loci of AAT were shown by AAT-1 and AAT-2. The phenotypes of AAT-1 were scored for each individual at the upper margin by B, C or BC. The relative positions of bands encoded by alleles *b* and *c* at the *AAT-1* locus were marked by b and c, respectively. The origin was shown by O.

Table 2. Developmental capacity in the crosses between females homozygous and males heterozygous at the *AAT-1* locus

Parents (Phenotype)		Total number of eggs	Number of normally cleaved eggs	Number of normally hatched tadpoles	Number of normally feeding tadpoles
Female	Male				
Hir. ♀ 1 (B)	Hir. ♂ 6 (BC)	199	178 (89.4%)	171 (85.9%)	155 (77.9%)
Hir. ♀ 1 (B)	Hir. ♂11 (BC)	201	193 (96.0%)	190 (94.5%)	177 (88.1%)
Hir. ♀ 2 (B)	Hir. ♂ 6 (BC)	202	180 (89.1%)	175 (86.6%)	167 (82.7%)
Hir. ♀ 2 (B)	Hir. ♂11 (BC)	219	215 (98.2%)	215 (98.2%)	200 (91.3%)

Table 3. Number of nucleoli in the offspring

Parents		No. of offspring examined	Offspring				
Female	Male		1-nu	2-nu	3-nu	1-nu/2-nu	2-nu/3-nu
Hir.♀1	Hir.♂ 6	149	57	85	4	2	1
Hir.♀1	Hir.♂11	158	72	84	2	0	0
Hir.♀2	Hir.♂ 6	170	91	79	0	0	0
Hir.♀2	Hir.♂11	187	93	94	0	0	0
Total (%)		664	313 (47.1)	342 (51.5)	6 (0.9)	2 (0.3)	1 (0.2)

and expected numbers of phenotypes in males, but there were in females ($\chi^2 = 29.5$, $P < 0.001\%$). If the *AAT-1* locus is supposed to be sex-linked and situated on the ZZ/ZW-type sex chromosomes of *B. buergeri*, the expected numbers of each phenotype coincide with the actual numbers (Table 1).

Two field-caught females (Hir.♀1, Hir.♀2) having phenotype B at the *AAT-1* locus were mated with two field-caught males (Hir.♂6, Hir.♂11) having phenotype BC at this locus (Table 2). Most of the eggs (89.1~98.2% of the total number of eggs) cleaved normally, 85.9~98.2% of the total number of eggs hatched normally, and 77.9~91.3% of the total number of eggs became normally feeding tadpoles (Table 2). The sex of each tadpole at TK stage X~XIII was determined by counting the number of nucleoli in the nucleus of individual cells (Table 3, Fig. 1). Of 664 offspring produced from four matings, 313 (47.1%) had single nucleolus per nucleus (1-nu; females), 342 (51.5%) had two nucleoli per nucleus (2-nu; males), and nine were 3-nu, 1-nu/2-nu mosaics or 2-nu/3-nu mosaics (Table 3). There were no differences between male and female tadpoles in viability. Analysis of the AAT-1 phenotypes

Table 4. Phenotypes at the *AAT-1* locus in the offspring

Parents (Phenotype)		No. of offspring examined	Offspring					
Female	Male		Phenotype	1-nu	2-nu	3-nu	1-nu/2-nu	2-nu/3-nu
Hir.♀1 (B)	Hir.♂ 6 (BC)	149	B	26	47	2	1	1
			C	31	0	0	0	0
			BC	0	38	2	1	0
Hir.♀1 (B)	Hir.♂11 (BC)	158	B	38	50	2	0	0
			C	34	0	0	0	0
			BC	0	34	0	0	0
Hir.♀2 (B)	Hir.♂ 6 (BC)	170	B	45	39	0	0	0
			C	46	0	0	0	0
			BC	0	40	0	0	0
Hir.♀2 (B)	Hir.♂11 (BC)	187	B	42	49	0	0	0
			C	50	0	0	0	0
			BC	1	45	0	0	0
Total		664	B	151	185	4	1	1
			C	161	0	0	0	0
			BC	1	157	2	1	0

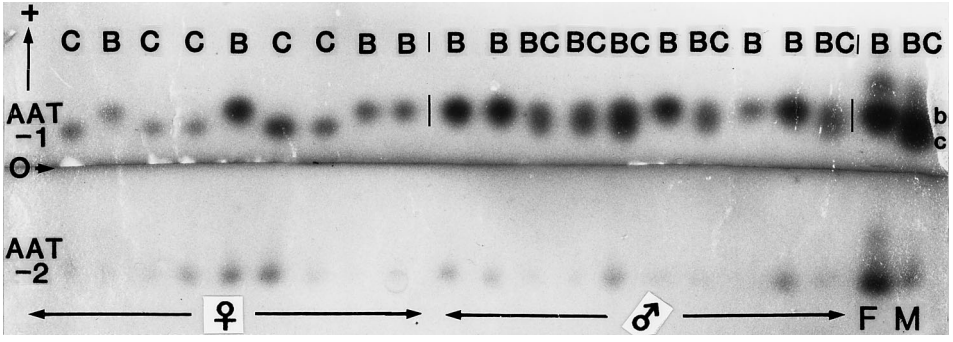

Fig. 3. Electrophoretic patterns of aspartate aminotransferase (AAT) in nine female and 10 male offspring and their female (F) and male (M) parents of *Buergeria buergeri*. The two loci of AAT were shown by AAT-1 and AAT-2. The phenotypes of AAT-1 were scored for each individual at the upper margin by B, C or BC. The relative positions of bands encoded by alleles *b* and *c* at the *AAT-1* locus were marked by b and c, respectively. The origin was shown by O.

(Table 4, Fig. 3) revealed that of 313 1-nu female tadpoles, 151 were B in phenotype, 161 were C in phenotype, and one was BC in phenotype. On the other hand, of 342 2-nu male tadpoles, 185 were B in phenotype, and 157 were BC in phenotype. Almost all females appeared to be *b* or *c* homozygotes, whereas there were nearly an equal number of *bb* homozygotes and *bc* heterozygotes in males. Thus it is evident that alleles on the Z chromosome of female parents are expressed, whereas those on the W chromosome are not.

DISCUSSION

Chromosome pair No. 7 of *Buergeria buergeri* are sex chromosomes of the ZZ/ZW type, and Z chromosome is a SAT-chromosome with a secondary constriction that corresponds to a nucleolar organizer (Ohta, 1986, 1987). The nucleolar organizer is situated on the Z chromosome, and a satellite is attached to the end of the long arm. On the other hand, neither the secondary constriction nor the satellite are observed in the W chromosome, making the distinction of W chromosome from Z chromosome easy. Because sex-determining genes are linked with a secondary constriction, males (ZZ) have two nucleolar organizers and females (ZW) have one nucleolar organizer (Ohta, 1986; Schmid *et al.*, 1993). The present study revealed that the *AAT-1* locus is sex-linked, and that its codominant alleles are expressed on the Z chromosome, but not on the W chromosome. Thus it is evident that the *AAT-1* locus of female offspring is hemizygous and that the allele present corresponds to that on the Z chromosome derived from the male parent. The mode of inheritance of the sex-linked *AAT-1* locus in *B. buergeri* is the same as for sex-linked loci in birds (Baverstock *et al.*, 1982) and butterflies (Johnson and Turner, 1979), in which sex-linked genes are carried exclusively on the Z or the X chromosome, respectively, resulting in hemizygous genes in the heterogametic sex. This is the first report of this mode of inheritance in amphibians.

As for sex-linked genes, several linkage relationships

between sex-determining genes and enzyme loci have been reported in amphibians. The *PEP-A* locus in *Pleurodeles waltl* was the first sex-linked gene detected (Ferrier *et al*., 1983). This locus was also demonstrated to be linked with sex-determining genes in the closely related *P. poireti* (Dournon *et al*., 1984). The *LDH-B* locus was identified as sex-linked in hybrids between female *Rana clamitans* and male *R. catesbeiana* (Elinson, 1981), as was the *ACON-1* locus in *Rana clamitans* (Elinson, 1983). In *Rana pipiens*, the *PEP-C* and *SOD-1* loci were demonstrated to be linked with sex-determining genes (Wright and Richards, 1983). These loci were also found to be linked with sex-determining genes in hybrids between female *R. pipiens* and male *R. palustris*, and the *SOD-1* locus was linked with sex-determining genes in hybrids between female *Rana sphenocephala* and male *R. blairi* (Wright *et al*., 1983). In hybrids between female *R. sphenocephala* and male *R. berlandieri*, in contrast, the *PEP-C* and *SOD-1* loci were not linked with sex-determining genes, although the *ADH-2* locus was found to be linked with the latter. All the loci clearly linked with the *ADH-2* locus, including the *Alb*, *PGM-1*, *F16DP* and β*-GLU* loci, were also linked with sex-determining genes in hybrids between female *Rana sphenocephala* and male *R. berlandieri*, and the *ACON-1* locus was linked with sex-determining genes in *R. sphenocephala* (Wright *et al*., 1983). The *mME* locus was reported to be sex-linked in *Xenopus laevis* (Graf, 1989). In the Okayama race of *R. porosa brevipoda*, sex-determining genes were found to be linked with the *MPI*, *LDH-B* and *PEP-B* loci on chromosome No. 4, whereas they were found to be linked with the *ME-1* locus on chromosome No. 3 in the Nagoya race of the same species (Nishioka and Sumida, 1994). The *MPI*, *SORDH*, *ENO* and *HK* loci on chromosome No. 4 were demonstrated to be linked with sex-determining genes in *R. nigromaculata* (Nishioka and Sumida, 1994). In *R. japonica*, the *Alb* locus was linked with sex-determining genes in the seven western populations, whereas the *MPI* locus was sex-linked in the two eastern populations. In the northwestern Akita population of *R. japonica*, none of the *Alb*, *AAT-1*, *ADA*, α*-GDH*, *LDH-B*, *ME-1*, *ME-2* and *MPI* loci were linked with sex-determining genes (Sumida and Nishioka, 1994). In the *Rana esculenta* group, the *MPI*, *LDH-B*, *HK-1* and *PEP-B* loci were reported to be sex-linked (Hotz *et al*., 1997). The present study demonstrated that the *AAT-1* locus is sex-linked in the Hiroshima population of *B. buergeri*, which has ZZ/ZW-type sex chromosomes. The sex-linked *AAT-1* locus has so far not been reported in any of the species of *Pleurodeles*, *Xenopus*, or *Rana*, listed above. Apparently the linkage relationships of sex-determining genes to other loci are evolutionarily unstable and there is no common ancestral or conserved sex-linkage group in amphibians.

Ohta (1986) found that chiasmata were clustered primarily near one end in lampbrush bivalent chromosome No. VII of *B. buergeri*, whereas they were almost evenly distributed along the total length in other bivalents. In the Hiroshima population of *B. buergeri*, lampbrush bivalent No. VII had a chiasma frequency of 0.43, whereas the other seven small bivalents had chiasma frequencies of 2.10~3.10. The homologues of each of the bivalents No. VII that lacked a chiasma were joined by one or two terminal fusions or at the small landmark situated at the site of 90.4% from one end of the bivalent. Some of the bivalents having only one chiasma were also joined by one or two terminal fusions or at the landmark. In any case, no chiasmata were observed within the range between the tip and about the middle of each bivalent No. VII (Ohta, 1986).

The present study revealed that of 313 1-nu females derived from four matings, almost all were hemizygous, *b* or *c*, at the *AAT-1* locus, but there was only one 1-nu female heterozygous at the *AAT-1* locus. This 1-nu heterozygous female is considered to be a recombinant produced by crossing-over between the sex-determining genes and the *AAT-1* locus at a rate of 0.15%. This result suggests that the *AAT-1* locus is situated near the nucleolar organizer on the Z chromosome and that this locus and the nucleolar organizer are recombined at an extremely low frequency, which agrees with the low chiasma frequency of lampbrush bivalent No. VII observed by Ohta (1986). In contrast, codominant AAT-1 alleles of heterozygous females are expressed both on the Z chromosome and on the W chromosome in the Kiriake population of Aomori Prefecture (Atsumi *et al*., unpublished). *Buergeria buergeri* from this population also has sex chromosomes of the ZZ/ZW type and the Z chromosome has both a secondary constriction and a satellite, while the W chromosome has neither (Ohta, unpublished). These preliminary data probably suggest that the *AAT-1* locus is situated not in the side of the satellite but of long arm.

The reasons why the AAT-1 allele on the W chromosome is not expressed are considered as follows: (1) the W chromosome is entirely heterochromatic and extensive genetic deterioration occurs to the heterochromatic region, (2) the AAT-1 gene is lost because of deletion, or (3) the expression of AAT-1 genes is inactivated by factors such as interaction with other genes. When triploids were produced from fertilized eggs of *B. buergeri* from the Hiroshima population by suppressing the extrusion of the second polar body, there were nearly an equal number of males and females among the ZZW triploids, whereas all ZWW triploids were females and all ZZZ triploids were males (Ohta, 1988). This suggests that the sex-determining genes on the W chromosome are probably functioning, and thus it is difficult to accept the first reason. Further examination at the gene level will be necessary to answer the question.

Dosage compensation of sex-linked genes is accomplished by random inactivation of one of the two X chromosomes in the homogametic sex in female mammals (Lyon, 1961; Ohno, 1967). According to Cayrol *et al*. (1983), Schmid *et al*. (1991) and Solari (1994), there are no indications of dosage compensation mechanisms in the homogametic (XX or ZZ) sex of amphibian species with recognizable, heteromorphic sex chromosomes as in birds, as suggested by Baverstock *et al*. (1982). It is also necessary to examine quantitatively the difference between males and females in the activity of the AAT-1 gene and to verify the dosage compen-

sation of AAT-1 genes in *Buergeria buergeri*. This species will serve as an excellent example for examining dosage compensation at the *AAT-1* locus in the homogametic sex.

ACKNOWLEDGMENTS

The authors thank Emeritus Professor M. Nishioka and the late Professor H. Ueda for their encouragement and valuable advice in this study. Thanks are also extended to Dr. T. Uzzell for his critical reviewing of the original manuscript.

REFERENCES

Baverstock PR, Adams M, Polkinghorne RW, Gelder M (1982) A sex-linked enzyme in birds—Z-chromosome conservation but no dosage compensation. Nature 296: 763–766

Cayrol C, Jaylet A, Ferrier V, Gasser F (1983) A genetic study of various enzyme polymorphisms in *Pleurodeles waltlii* (Urodele, Amphibian). III. The relationship between sex-linked peptidase-1 expression and gene-dose effects. Biochem Genet 21: 551–559

Chang CY, Witschi E (1955) Breeding of sex-reversed males of *Xenopus laevis* Daudin. Proc Soc Exp Biol Med 89: 150–152

Chang CY, Witschi E (1956) Gene control and hormonal reversal of sex differentiation in *Xenopus*. Proc Soc Exp Biol Med 93: 140–144

Dournon C, Guillet F, Boucher D, Lacroix JC (1984) Cytogenetic and genetic evidence of male sexual inversion by heat treatment in the newt *Pleurodeles poireti*. Chromosoma 90: 261–264

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

Elinson RP (1983) Inheritance and expression of a sex-linked enzyme in the frog, *Rana clamitans*. Biochem Genet 21: 435–442

Engel W, Schmid M (1981) H-Y antigen as a tool for the determination of the heterogametic sex in Amphibia. Cytogenet Cell Genet 30: 130–136

Ferrier V, Gasser F, Jaylet A, Cayrol C (1983) A genetic study of various enzyme polymorphisms in *Pleurodeles waltlii* (Urodele Amphibian). II. Peptidases: demonstration of sex linkage. Biochem Genet 21: 535–549

Gallien L (1953) Inversion totale du sexe chez *Xenopus laevis* Daud. à la suite d'un traitement gynogène par le benzoate d'oestradiol, administré pendant la vie larvaire. C R Acad Sc Paris Sér D 237: 1565–1566

Graf J-D (1989) Sex-linkage of malic enzyme in *Xenopus laevis*. Experientia 45: 194–196

Harris H, Hopkinson DA (1976) Handbook of Enzyme Elecrophoresis in Human Genetics. 1st ed, North-Holland, Amsterdam

Hillis DM, Green DM (1990) Evolutionary changes of heterogametic sex in the phylogenetic history of amphibians. J Evol Biol 3: 49–64

Hotz H, Uzzell T, Berger L (1997) Linkage groups of protein-coding genes in western Palearctic water frogs reveal extensive evolutionary conservation. Genetics 147: 255–270

Humphrey RR (1942) Sex of the offspring fathered by two *Ambystoma* females experimentally converted into males. Anat Rec 82 (Suppl 77): 469

Humphrey RR (1945) Sex determination in ambystomid salamanders: A study of the progeny of females experimentally converted into males. Am J Anat 76: 33–36

Humphrey RR (1957) Male homogamety in the Mexican axolotl: A study of the progeny obtained when germ cells of a genetic male are incorporated in a developing ovary. J Exp Zool 134: 91–101

Johnson MS, Turner JRG (1979) Absence of dosage compensation for a sex-linked enzyme in butterflies (*Heliconius*). Heredity 43: 71–77

Kawamura T, Nishioka M (1977) Aspects of the reproductive biology of Japanese anurans. In "The Reproductive Biology of Amphibians" Ed by DH Taylor, SI Guttman, Plenum Press, New York, pp 103–139

Lyon MF (1961) Gene action in the X-chromosome of the mouse (*Mus musculus* L.). Nature 190: 372–373

Nardi I, Andronico F, de Lucchini S, Batistoni R (1986) Cytogenetics of the European plethodontid salamanders of the genus *Hydromantes* (Amphibia, Urodela). Chromosoma 94: 337–388

Nishioka M, Ohtani H, Sumida M (1980) Detection of chromsomes bearing the loci for seven kinds of proteins in Japanese pond frogs. Sci Rep Lab Amphibian Biol Hiroshima Univ 4: 127–184

Nishioka M, Sumida M (1994) 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, Hanada H, Miura I, Ryuzaki M (1994) Four kinds of sex chromosomes in *Rana rugosa*. Sci Rep Lab Amphibian Biol Hiroshima Univ 13: 1–34

Ohno S (1967) Sex Chromosomes and Sex-linked Genes. 1st ed, Spring-Verlag, New York

Ohta S (1986) Sex-determining mechanism in *Buergeria buergeri* (Schlegel) I. Heterozygosity of chromosome pair No. 7 in the female. Sci Rep Lab Amphibian Biol Hiroshima Univ 8: 29–43

Ohta S (1987) Sex-determining mechanism in *Buergeria buergeri* (Schlegel) II. The effects of sex hormones on the differentiation of gonads and the offspring of sex-reversed females. Sci Rep Lab Amphibian Biol Hiroshima Univ 9: 213–238

Ohta S (1988) Triploids and gynogenetic diploids in *Buergeria buergeri*. Jpn J Genet 63: 588 (in Japanese)

Ponse K (1942) Sur la digametie du crapaud hermaphrodite. Rev Suisse Zool 49: 185–189

Schempp W, Schmid M (1981) Chromosome banding in Amphibia. VI. BrdU-replication pattern in Anura and demonstration of XX/XY sex chromosomes in *Rana esculenta*. Chromosoma 83: 697–710

Schmid M (1980) Chromosome banding in Amphibia. V. Highly differentiated ZW/ZZ sex chromosomes and exceptional genome size in *Pyxicephalus adspersus* (Anura, Ranidae). Chromosoma 80: 69–96

Schmid M, Olert J, Klett C (1979) Chromosome banding in Amphibia III. Sex chromosome in *Triturus*. Chromosoma 71: 29–55

Schmid M, Haaf T, Geile B, Sims S (1983) Chromosome banding in Amphibia. VIII. An unusual XY/XX-sex chromosome system in *Gastrotheca riobambae* (Anura, Hylidae). Chromosoma 88: 69–82

Schmid M, Steinlein C, Feichtinger W, de Almeida CG, Duellman WE (1988) Chromosome banding in Amphibia. XIII. Sex chromosomes, heterochromatin and meiosis in marsupial frogs (Anura, Hylidae). Chromosoma 97: 33–42

Schmid M, Steinlein C, Feichtinger W (1989) Chromosome banding in Amphibia. XIV. The karyotype of *Centrolenella antisthenesi* (Anura, Centrolenidae). Chromosoma 97: 434–438

Schmid M, Steinlein C, Freiedl R, de Almedia CG, Haaf T, Hillis DM, Duellman WE (1990) Chromosome banding in Amphibia. XV. Two types of Y chromosomes and heterochromatin hypervariability in *Gastrotheca pseustes* (Anura, Hylidae). Chromosoma 99: 413–423

Schmid M, Steinlein C (1991) Chromosome banding in Amphibia. XVI. High-resolution replication banding patterns in *Xenopus laevis*. Chromosoma 101: 123–132

Schmid M, Nanda I, Steinlein C, Kausch K, Epplen JT, Haaf T (1991) Sex-determining mechanisms and sex chromsomes in amphibia. In "Amphibian Cytogenetics and Evolution" Ed by DM Green, SK Sessions, Academic Press, San Diego, pp 393–430

Schmid M, Ohta S, Steinlein C, Guttenbach M (1993) Chromosome

banding in Amphibia XIX. Primitive ZW/ZZ sex chromosomes in *Buergeria buergeri* (Anura, Rhacophoridae). Cytogenet Cell Genet 62: 238–246

Sims SH, Macgregor HC, Pellatt PS, Horner HA (1984) Chromosome 1 in crested and marbled newts (*Triturus*): An extraordinary case of heteromorphism and independent chromosome evolution. Chromosoma 89: 169–185

Solari AJ (1994) Sex Chromosomes and Sex Determination in Vertebrates. 1st ed, CRC Press, London

Stephenson EM, Robinson ES, Stephenson NG (1972) Karyotype variation within the genus *Leiopelma* (Amphibia, Anura). Can J Genet Cytol 14: 691–702

Sumida M, Nishioka M (1994) Geographic variability of sex-linked loci in the Japanese brown frog, *Rana japonica*. Sci Rep Lab Amphibian Biol Hiroshima Univ 13: 173–195

Taylor AC, Kollros JJ (1946) Stages in the normal development of *Rana pipiens* larvae. Anat Rec 94: 7–24

Ueda H (1990) Offspring of sex-reversed males in *Bufo viridis* Laur. Sci Rep Lab Amphibian Biol Hiroshima Univ 10: 155–164

Wachtel SS, Koo GC, Boyse EA (1975) Evolutionary conservation of H-Y (male) antigen. Nature 254: 270–272

Wright DA, Richards CM (1983) Two sex-linked loci in the leopard frog, *Rana pipiens*. Genetics 103: 249–261

Wright DA, Richards CM, Frost JS, Camozzi AM, Kunz BJ (1983) Genetic mapping in amphibians. In “Isozymes: Current Topics in Biological and Medical Research Vol 10” Alan R Liss, New York, pp 287–311

(Received April 6, 1998 / Accepted May 12, 1998)