EVIDENCE FOR A POLYMORPHISM IN GAMETIC SEGREGATION USING A MALATE DEHYDROGENASE LOCUS IN THE TETRAPLOID TREEFROG *HYLA VERSICOLOR*[1]

ROY G. DANZMANN AND JAMES P. BOGART

Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Manuscript received June 12, 1981
Revised copy accepted October 19, 1981

ABSTRACT

Artificial cross combinations of tetraploid *Hyla versicolor* were analyzed electrophoretically using a polymorphic malate dehydrogenase locus (MDH-1) to determine the mechanism of chromosome segregation. Models for differentiating between disomic and tetrasomic inheritance are presented and tested. In some crosses progeny genotypes fit a disomic mode of segregation. In other crosses there is only evidence for a tetrasomic mode of segregation. Additional crosses produced genotypic ratios which conformed to either a disomic or tetrasomic mode of segregation. The same type of inheritance was demonstrated for any individual when used in multiple cross combinations. These results suggest that there exists in *H. versicolor* a polymorphism with respect to segregation of gametes, resulting from differences in chromosome pairings during meiosis I.

THE incidence of polyploidy in fish, amphibians, and reptiles has been reviewed (BOGART 1980; SCHULTZ 1980). Unlike certain families of fish, whose major lineages may be derived from distant polyploid events (UYENO and SMITH 1972; BAILEY, POULTER and STOCKWELL 1978), many polyploid species of frogs have closely related diploid species which form diploid-polyploid cryptic species pairs (BEÇAK, BEÇAK and VIZOTTO 1970; BOGART and WASSERMAN 1972; BATISTIC *et al.* 1975; BOGART and TANDY 1976; BOGART 1980; BARRIO 1980). In most cases the diploid and polyploid cryptic species of amphibians are morphologically similar and specific designations have not been formally recognized.

The North American treefrogs *Hyla chrysoscelis* and *H. versicolor* are such a diploid-tetraploid cryptic species pair. *H. versicolor* has 48 chromosomes (WASSERMAN 1970) and almost twice the DNA of 24-chromosome *H. chrysoscelis*, but both species possess similar amounts of RNA (BACHMANN and BOGART 1975). The overall similarity of the two species and presence of quadrivalents during meiosis in *H. versicolor* (BOGART and WASSERMAN 1972) suggest that *H. versicolor* is an autopolyploid, but the actual classification of this species as an auto- or allopolyploid is not firmly established. *H. versicolor* may have arisen through hybridization in a zone of secondary contact between genetically distinct populations of *H. chrysocelis*, or the presence of *H. versicolor* may have

[1] This work was supported by NSERC (Canada) grant A9866.

subsequently isolated populations of *H. chrysoscelis* (BOGART and WASSERMAN 1972). Eastern and western *H. chrysoscelis* populations are reported to differ in vocalization (GERHARDT 1974; RALIN 1977) and allele frequencies (RALIN and SELANDER 1979). In addition, MAXSON, PEPPER and MAXSON (1977) reported immunological distance data, indicating genetic differences between eastern and western populations of *H. chrysoscelis*. Furthermore, these authors state that these populations have maintained separate gene pools for approximately four million years. They also suggest that *H. versicolor* arose as a result of allotetraploidy involving eastern and western *H. chrysoscelis*. RALIN (1978) contested the interpretation of MAXSON, PEPPER and MAXSON's immunological data however, and justified the need for additional information.

Distinguishing between an autopolyploid or an allopolyploid ancestry using chromosome associations is difficult, due to the confounding nature of chromosome diploidization (formation of two bivalents from a tetravalent through time), and possible chromosome homology differences and/or gene regulation factors determining chromosome pairing. *H. versicolor* provides a unique opportunity to investigate these possibilities through the examination of chromosome segregation using isozyme markers.

Studies with polyploid fishes demonstrate a general loss of duplicate gene expression which has probably occurred during diploidization of the polyploid genome (ALLENDORF 1978; BAILEY, POULTER and STOCKWELL 1978; FERRIS, PORTNOY and WHITT 1979). To date only disomic segregation, resulting from diploidized loci still retaining duplicate gene expression, has been demonstrated in fish with a polyploid ancestry (ALLENDORF and UTTER 1973; ALLENDORF 1975; MAY 1975; MAY, WRIGHT and STONEKING 1979).

Reports of tetrasomic inheritance for blood albumins in a South American frog *Odontophrynus americanus* (BEÇAK, SCHWANTES and SCHWANTES 1968) and various enzymes in the salmonid fish: *Coregonus lavaretus*; *Salmo gairdneri*; and *Salvelinus fontinalis* (WOLF, ENGEL and FAUST 1970; ENGEL, OP'T HOF and WOLF 1970; STEGEMAN and GOLDBERG 1972), were based on examination of population phenotypes and, as pointed out by ALLENDORF and UTTER (1973), may be in error since population phenotypes are not a valid criterion upon which to base the existence of tetrasomic inheritance. Later studies, using artificial crosses, affirmed the disomic, rather than tetrasomic nature of chromosome segregation in the rainbow trout (*S. gairdneri*) (ROPERS, ENGEL, and WOLF 1973; ENGEL, SCHMIDTKE and WOLF 1975). *H. versicolor* is a more recent tetraploid and might be expected to demonstrate tetrasomic inheritance.

This study investigates chromosome segregation in *H. versicolor* using isozyme variation at a malate dehydrogenase locus (MDH; E.C. 1.1.1.37) from liver tissue. This enzyme is dimeric in structure in *H. versicolor* and other hylid frogs which have been investigated (RALIN and SELANDER 1979; GERHARDT, GUTTMAN and KARLIN 1980), and exhibits duplicate gene expression in *H. versicolor* (RALIN and SELANDER 1979). The use of duplicate gene expression to determine allelic segregation at a locus has been established (ALLENDORF and UTTER 1973, 1976; ALLENDORF 1975; ALLENDORF, UTTER and MAY 1975).

Crosses were performed to determine the mode of inheritance of MDH-1 allozymes in *H. versicolor*, and therefore to assess the nature of chromosome association at meiosis I in this tetraploid species. The results of these crosses are reported in this paper.

MATERIALS AND METHODS

The parents used were obtained from several distant localities to maximize the possibility of obtaining different alleles (Table 1). Ova and sperm were obtained using standard techniques (RUGH 1948; MECHAM 1965; BOGART and BOGART 1971). The females were either induced to ovulate by injection of pituitary gland suspensions (from *Bufo americanus*) or were captured in amplexus close to the laboratory and used for crosses the same night.

In the first series of crosses (78–3, –6, –13; and 79–3, –5, –6) (Table 1) the male parent was randomly selected, but in the later crosses the males were electrophoretically screened for MDH. Isozymes of this enzyme were detected from the homogenate of a single toe. Symmetrically heterozygous individuals or triallelic individuals were preferred for the crosses, since gametic segregation in these genotypes differentiate between a disomic or tetrasomic mode of inheritance. Segregation involving asymmetrical heterozygotes provide the same gametic ratios with either a disomic or tetrasomic mode of inheritance (ALLENDORF, UTTER and MAY 1975). Reproductively mature females are more difficult to obtain than males, because they cannot be located by vocalizations during the breeding season. Consequently, the probability of obtaining a female with a symmetrically heterozygous genotype is low. Therefore, this study predominantly provides information of gametic segregation in *H. versicolor* males.

Symmetrically heterozygous genotypes possess double doses of two different allelic variants. Such genotypes have been referred to as duplex. Asymmetrical heterozygotes possess three doses of one allele and one dose of a variant allele. Such genotypes have previously been designated as triplex for a dominant allele expressing three gene doses, and simplex for the alternate recessive allele if it expresses three gene doses (MATHER 1936; BURNHAM 1962).

Eggs and tadpoles were maintained in filtered pond water for about one month then moved to aged tap water. They were fed boiled lettuce and raised until most of them exhibited hind limbs (Stage XI) until metamorphosis and complete tail resorption (Stage XXV) from RUGH (1948). Tadpoles attaining these developmental stages were randomly selected for electrophoresis.

Karyology: Considering the morphological similarity of *H. versicolor* and *H. chrysoscelis*, it was necessary to confirm the identity of the parents chromosomally. Chromosomes were obtained from the parents and two to five tadpoles in each cross using previous techniques (BOGART 1967).

Electrophoresis: Livers (including pancreas) obtained from tadpoles were frozen in deionized water and stored at —70° to —90° for a few days to several weeks. Just prior to electrophoresis, the tissues were partially thawed and homogenized with a glass hand homogenizer. Liver homogenate was applied directly to the gel using Whatman No. 3 filter paper wicks.

Horizontal starch gel electrophoresis following UTTER, HODGINS and ALLENDORF (1974) was performed using an amino-propyl morpholine buffer at pH 6.5 (CLAYTON and TRETIAK 1972) for separation of MDH isozymes. Electrophoretic loci were numbered from anode to cathode. The most common MDH allozyme was arbitrarily assigned an electrophoretic mobility of 100 and all other allozymes at the locus with a value relative to the most common allozyme and the origin. An allozyme migrating half the distance of the most common allozyme would have a value of 50. This scoring system is valid only for the buffer system on which the enzyme was originally assayed as relative mobilities vary with the pH of the buffer system.

Models Tested: Tetrasomic inheritance involves random segregation of all four homologs and may be cytologically recognized by the formation of tetravalents in Meiosis I. The gametic ratios produced from a symmetrically heterozygous individual would be 1:4:1 (see Figure 1). If segregation was disomic then either homozygous or heterozygous bivalents could result in a symmetrically heterozygous MDH phenotype. Homozygous bivalents segregate only heterozygous gametes (Figure 2: upper), while heterozygous bivalents would produce homozygous : heterozygous : alternate homozygous gametes in a 1:2:1 ratio (Figure 2: lower).

TABLE 1

Sources of the two parents used for each cross

Cross no.	Population–Female*	Population–Male
78–6	2–4491†	2–4489
78–13	2–4491	2–4488
79–12	2–4891	10–4877
79–14	6–4897	6–4898
79–15	6–4897	6–4899
79–17	2–4928	6–4929
79–18	2–4928	5–4930
79–19	2–4928	5–4931
79–20	1–4932	9–4933
79–21	1–4932	6–4934
80–1	1–010‡	1–011
80–3	1–002	1–011
80–10	4–017	4–005
80–11	1–006	1–014
80–13	1–004	1–014
80–18	3–023	5–008
80–19	5–016	5–008
80–20	1–009	5–008
80–21	1–001	5–008
80–24	1–003	6–005
80–26	1–007	6–031
80–27	1–035	6–023
80–29	1–007	6–023
80–30	1–008	6–023
80–31	1–035	6–031
80–33	1–007	6–017

* Parents are listed according to their population of origin and individual catalog number by the first and second numbers respectively (e.g. population-individual). The population localities, with latitude and longitude are: (1) Aberfoyle, Ontario (43°28′ 80°09′); (2) Guelph, Ontario (43°33′ 80°15′); (3) Guelph, Ontario (43°27′ 80°13′); (4) Caledon East, Ontario (43°50′ 79°55′); (5) Port Perry, Ontario (44°06′ 78°57′); (6) Rosseau, Ontario (45°14′ 79°39′); (9) Lake Riviera, Manitoba (49°40′ 96°34′); (10) McDade, Texas (30°17′ 97°15′).

† Parents used in 78– and 79– crosses are catalog numbers of JPB.

‡ Parents used in 80– crosses are catalog numbers of RGD. These specimens will be deposited in the collection of amphibians and reptiles in the Department of Ichthyology and Herpetology at the Royal Ontario Museum.

The observed electrophoretic phenotypes (and presumed genotypes) of the progeny were compared with Mendelian expectations derived from the presumed parental genotypes. Probabilities derived from Chi-square tests (using YATES's correction for continuity) performed on the observed and expected genotypic classes assessed the various inheritance models in *H. versicolor*.

RESULTS

Phenotypes observed: Twelve MDH phenotypes were observed in progeny from various cross combinations. In one cross (80–1) all 12 phenotypes were observed as both parents in each expressed three allozymes representing four alleles. These alleles were designated MDH-1(100), (48), (134), and (142), according to mobility (Figure 3). Most crosses involved or produced only

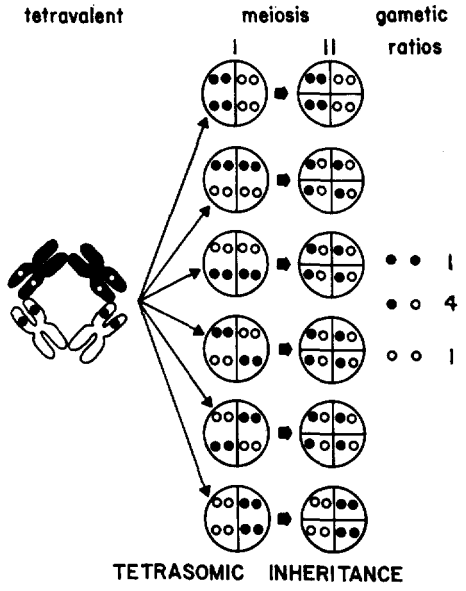

FIGURE 1.—In tetrasomic inheritance, the four homeologous chromosomes in a symmetrically heterozygous parent (2 black and 2 white "alleles") associate in a tetrad. If the association and subsequent segregation is random, three classes of gametes are produced in the ratio of 1 homozygous (black "alleles") to 4 heterozygous (black and white "alleles") to 1 homozygous (white "alleles").

those phenotypes shown in Figure 4. MDH-1(100) is probably the same allele as MDH-1(83) described by RALIN and SELANDER (1979), as this was the most common allozyme encountered in their investigation. Since the buffer system used by RALIN and SELANDER (1979) was not indicated, it is difficult to relate their additional allozyme mobilities to our study. Therefore, it was necessary to redesignate the phenotypes observed according to the CLAYTON and TRETIAK buffer system. A cathodal zone of weak staining intensity was found in certain individuals, that probably corresponds to the MDH-3 isozyme of RALIN and SELANDER (1979). This isozyme was not consistently reproduced in these same individuals when re-examined and probably represents products of enzyme degradation or fragmentation. GERHARDT, GUTTMAN and KARLIN (1980), using a Tris-citrate pH 8.0 buffer system, observed only two MDH loci in *Hyla cinerea* and *Hyla gratiosa*, with a polymorphism at the MDH-1 locus, which is consistent with our results.

Phenotypes a,b,k and l in Figure 3 and k and m in Figure 4 indicate that three alleles can be present in one individual, while Figure 3:j suggests that four separate alleles produced the observed staining intensity. These phenotypes clearly show duplicate gene expression at the MDH-1 locus. Relative electrophoretic staining intensities also suggest duplicate gene expression as the asymmetrical heterozygotes with presumed 3:1 or 1:3 gene dosages approximate 9:6:1 or 1:6:9 staining intensities respectively, for a dimeric enzyme (Figure 3:c, d and h: Figure 4:b, c, d, e and n) (BAILEY *et al.* 1970; ALLENDORF, UTTER

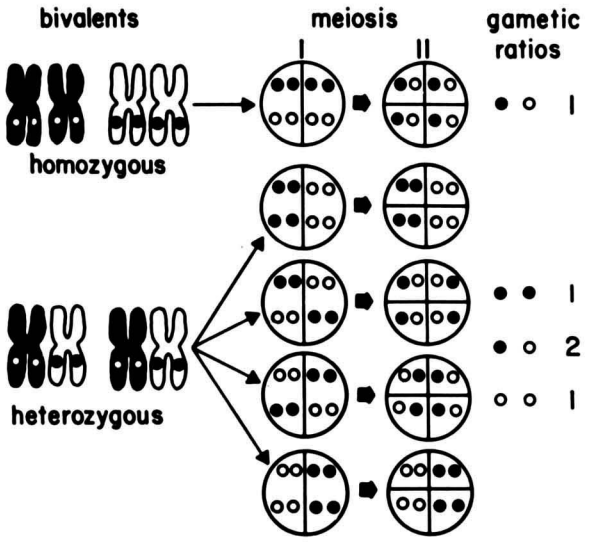

FIGURE 2.—In disomic inheritance, the four homeologous chromosomes in a symmetrically heterozygous parent (2 black and 2 white "alleles") pair and form dyads. If the homeologs paired such that the alleles are homozygous in each dyad (upper), then only heterozygous gametes would be produced. If the homeologs paired as heterozygous dyads (lower), three classes of gametes would be produced in the ratio of 1 homozygous (black "alleles") to 2 heterozygous (black and white "alleles") to 1 homozygous (white "alleles").

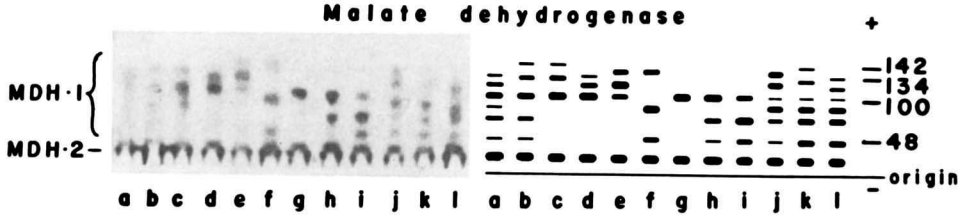

FIGURE 3.—Progeny phenotypes produced from cross 80-1. Homodimeric bands for MDH-1 are indicated to the right of the zymogram. The presumed genotypes are: MDH-1$^{(134/100^2/48)}$ (a); MDH-1$^{(142/100^2/48)}$ (b); MDH-1$^{(142/100^3)}$ (c); MDH-1$^{(134/100^3)}$ (d); MDH-1$^{(142/134/100^2)}$ (e); MDH-1$^{(142/134/48^2)}$ (f); MDH-1$^{(100^4)}$ (g); MDH-1$^{(100^3/48)}$ (h); MDH-1$^{(100^2/48^2)}$ (i); MDH-1$^{(142/134/100/48)}$ (j); MDH-1$^{(142/100/48^2)}$ (k) and MDH-1$^{(134/100/48^2)}$ (l).

and MAY 1975). It is apparent therefore, that the MDH-1 isozyme is coded at duplicate loci and each allozyme has equal staining intensity.

Inheritance studies: Segregation in 72 genomes of *H. versicolor* was examined through 26 artificial cross combinations, in which the genotypes of 2,348 progeny were scored. Table 2 outlines the progeny ratios expected for the two most common types of crosses performed. These involved symmetrically heterozygous males and either homozygous or asymmetrically heterozygous females. The results from the various cross combinations are shown in Table 3. Tables 3 and 4

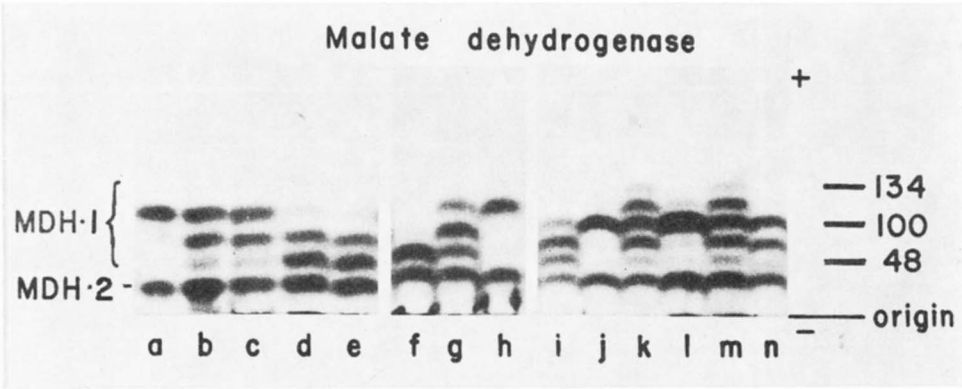

FIGURE 4.—Homodimeric bands for MDH-1 are indicated to the right of the gels. The presumed genotypes are: MDH-1$^{(100^4)}$ (a, h, j, l); MDH-1$^{(100^3/48)}$ (b, c, n); MDH-1$^{(100/48^3)}$ (d, e); MDH-1$^{(100^2/48^2)}$ (g, i); MDH-1$^{(48^4)}$ (f); and MDH-1$^{(100^2/48/134)}$ (k, m); a to e are from tadpoles (cross 79–18); f to n are from parents. MDH-2 is monomorphic.

TABLE 2

Expected progeny phenotypes from intraspecific crosses

Female genotype	Male genotype	Gametes produced Female		Male	Progeny phenotypes	Expected ratio* D Homo† II	Het† II	T
A^4‡	$A^2A'^2$	A^2	×	A^2	A^4		1	1
				AA'	A^3A'	1	2	4
				A'^2	$A^2A'^2$		1	1
A^3A'	$A^2A'^2$	A^2	×	A^2	A^4		1	1
		AA'		AA'	A^3A'	1	3	5
				A'^2	$A^2A'^2$	1	3	5
					AA'^3		1	1

* D = expected with disomic inheritance.
T = expected with tetrasomic inheritance.
† Homo II = homozygous bivalents.
Het II = heterozygous bivalents.
‡ MDH-1 allozymes are represented according to the following symbols: MDH-1 (100) = A
(48) = A′

represent the most common allozyme (100) as A, the second most common allozyme (48) as A′, and the third and fourth most common allozymes (134) and (142) as A″ and A‴ respectively.

From our results, crosses 79–14, 79–21, 80–18, 80–19, 80–20, 80–21, 80–24, and 80–27 suggest a disomic mode of segregation. Crosses 79–15, 79–18, 80–1, 80–10, 80–13, 80–26, 80–31, and 80–33 suggest a tetrasomic mode of segregation, while crosses 79–19, 80–3, 80–11, 80–29, and 80–30 suggest that both models are equally probable. Only crosses 80–13, 80–18, 20–19, and 80–21 significantly exclude the alternate hypotheses of disomic or tetrasomic inheritance ($p < 0.001$). There is no clear evidence to exclude either a disomic or tetrasomic mode

TABLE 3

Observed and expected segregation of MDH-1* *allozymes in cross combinations*

Cross	Parental phenotypes (presumed genotypes)		Progeny phenotypes: observed (expected with disomic inheritance) /expected with tetrasomic inheritance/							
	Female	Male	AAAA	AAAA′	AAA′A′	AA′A′A′	A″AAA	A″AAA′	χ^2 (df)	Probability
78–6	AAAA′	AAAA	47	55						
			/(51)/	/(51)/					0.48(1)	0.488
78–13	AAAA′	AAAA′	4	12	1					
			/(4.25)/	/(8.5)/	/(4.25)/				2.85(2)	0.240
79–12	AAAA′	A″AAA	1	5			5	4		
			/(3.75)/	/(3.75)/			/(3.75)/	/(3.75)/	1.67(3)	0.644
79–14	AAAA	AAA′A′	4	3	1					
			(2)	(4)	(2)				1.31(2)	0.519
			/1.33/	/5.33/	/1.33/				4.19(2)	0.123
79–15	AAAA	AAA′A′	2	6	1					
			(2.25)	(4.50)	(2.25)				0.50(2)	0.779
			/1.5/	/6.0/	/1.5/				0.04(2)	0.980
79–17	AAAA′	AA′A′A′		9	11	2				
				/(5.50)/	/(11)/	/(5.50)/			3.29(2)	0.193
79–18	AAAA′	AAA′A′	11	62	53	11				
			(17.13)	(51.38)	(51.38)	(17.13)			5.72(3)	0.126
			/11.42/	/57.08/	/57.08/	/11.42/			0.57(3)	0.903
79–19	AAAA′	AAA′A′	1	4	5	1				
			(1.38)	(4.13)	(4.13)	(1.38)			0.09(3)	0.993
			/0.92/	/4.58/	/4.58/	/0.92/			0.39(3)	0.943
79–20	AAAA	A′A′A′A′			113				—	1.0
79–21	AAAA	AAA′A′	4	6	3					
			(3.25)	(6.50)	(3.25)				0.04(2)	0.980
			/2.17/	/8.67/	/2.17/				1.41(2)	0.494

TABLE 3—Continued

Cross	Female	Male	AAAA	AAAA′	AAA′A′	AA′A′A′	A″AAA	A″AAA′	χ^2 (df)	Probability
80–03	AAAA	A‴AAA′	41	69			55	48		
			(53.25)	(53.25)			(53.25)	(53.25)	7.41(3)	0.059
			/35.50/	/71/			/71/	/35.50/	8.17(3)	0.042
80–10	AAAA	AAA′A′	12	45	16					
			(18.25)	(36.50)	(18.25)				3.73(2)	0.155
			/12.20/	/48.60/	/12.20/				5.73(2)	0.580
80–11	AAAA	AAA′A′	12	54	24					
			(22.50)	(45)	(22.50)				6.09(2)	0.048
			/15/	/60/	/15/				5.73(2)	0.057
80–13	AAAA	AAA′A′	16	77	17					
			(27.50)	(55)	(27.50)				16.44(2)	<0.001
			/18.30/	/73.30/	/18.30/				0.35(2)	0.839
80–18	AAAA	AAA′A′	20	27	21					
			(17)	(34)	(17)				2.33(2)	0.312
			/11.30/	/45.30/	/11.30/				20.43(2)	<0.001
80–19	AAAA	AAA′A′	27	39	29					
			(23.75)	(47.50)	(23.75)				2.61(2)	0.271
			/15.80/	/63.30/	/15.80/				26.40(2)	<0.001
80–20	AAAA′	AAA′A′	16	43	57	21				
			(17.10)	(51.40)	(51.40)	(17.10)			2.42(3)	0.466
			/11.40/	/57.10/	/57.10/	/11.40/			11.98(3)	0.007
80–21	AAAA	AAA′A′	23	29	17					
			(17.25)	(34.50)	(17.25)				2.32(2)	0.313
			/11.50/	/46/	/11.50/				18.61(2)	<0.001

TABLE 3—Continued

Cross	Female	Male	AAAA	AAAA′	AAA′A′	AA′A′A′	A″AAA	A″AAA′	χ^2 (df)	Probability
80–24	AAAA	AAA′A′	50	113	47					
			(52.50)	(105)	(52.50)				1.09(2)	0.580
			/35/	/140/	/35/				14.80(2)	0.001
80–26	AAAA′	AAA′A′	5	41	31	3				
			(10)	(30)	(30)	(10)			9.93(3)	0.019
			/6.60/	/33.30/	/33.30/	/6.60/			3.29(3)	0.335
80–27	AAAA	AAA′A′	6	25	14					
			(11.25)	(22.50)	(11.25)				2.63(2)	0.268
			/7.50/	/30/	/7.50/				5.61(2)	0.061
80–29	AAAA′	AAA′A′	15	47	41	7				
			(13.75)	(41.25)	(41.25)	(13.75)			3.55(3)	0.302
			/9.17/	/45.83/	/45.83/	/9.17/			3.53(3)	0.305
80–30	AAAA	AAA′A′	40	106	31					
			(44.25)	(88.50)	(44.25)				7.25(2)	0.027
			/29.50/	/118/	/29.50/				4.54(2)	0.103
80–31	AAAA	AAA′A′	3	25	2					
			(7.50)	(15)	(7.50)				11.48(2)	0.003
			/5/	/20/	/5/				2.71(2)	0.258
80–33	AAAA′	AAA′A′	9	60	39	8				
			(14.50)	(43.50)	(43.50)	(14.50)			10.46(3)	0.015
			/9.70/	/48.30/	/48.30/	/9.50/			4.35(3)	0.219

* MDH-1 allozymes are represented according to the following symbols: MDH-1 (100) = A
(48) = A′
(134) = A″
(142) = A‴

† Assuming no crossing over between chromatids.

TABLE 4

Observed and expected segregation of MDH-1 *allozymes in cross 80-1 ($A''A^2A'$) × ($A'''A^2A'$)*

	Progeny phenotypes: observed (expected with disomic inheritance) /expected with tetrasomic inheritance/ /(expected with disomic and tetrasomic inheritance)/										
Models*	AAAA	A'''A''AA	A'''AAA and A''AAA	A'''A''AA'	A'''AAA' and A''AAA'	AAAA'	A'''AA'A' and A''AA'A'	AAA'A'	A'''A''A'A'	χ^2 (df)	Probability
	7	32	37	25	68	37	33	26	13		
A	(17.37)	(17.37)	(34.75)	(34.75)	(69.50)	(34.75)	(34.75)	(17.37)	(17.37)	24.46(8)	0.002
B & C	—	(34.75)	(34.75)	(34.75)	(69.50)	(34.75)	(34.75)	(34.75)	—	—	<0.001
D	—	(69.50)	—	—	(139)	—	—	(69.50)	—	—	<0.001
E	/7.70/	/30.90/	/30.90/	/30.90/	/77.20/	/30.90/	/30.90/	/30.90/	/7.70/	7.67(8)	0.466
F & H	/(11.60)/	/(23.20)/	/(34.75)/	/(34.75)/	/(69.50)/	/(34.75)/	/(34.75)/	/(23.20)/	/(11.60)/	7.41(8)	0.493
G & I	—	/(46.30)/	/(23.20)/	/(23.20)/	/(92.70)/	/(23.20)/	/(23.20)/	/(46.30)/	—	—	<0.001

* The models tested are outlined in Table 5.

of segregation in crosses 79–14, 79–15, 79–18, 79–19, 79–21, 80–1, 80–3, 80–10, 80–11, 80–20, 80–24, 80–26, 80–27, 80–29, 80–30, 80–31, and 80–33. Crosses 78–6, 78–13, 79–12, 79–17, and 79–20 predict the same gametic ratios with either a disomic or tetrasomic mode of inheritance and cannot be used to test either model, but progeny ratios observed from these crosses are consistent with Mendelian expectations.

The results from cross 80–1 involving a triallelic female and male are shown in Table 4. Four allozymes were present in the progeny from this cross, but only three are shown in Table 4. Allozymes (134) and (142) are very similar in mobility on a CLAYTON and TRETIAK pH 6.5 buffer system. Since it was difficult to distinguish between phenotypes involving these two alleles (Figure 3) they were represented as a single class in Table 4. The various models tested in this cross are outlined in Table 5.

In all except three of the crosses, the mode of inheritance shown by each male was identical. For instance, male 1–011 demonstrated a disomic and/or tetrasomic mode of segregation in cross 80–1 and 80–3. Male 1–014 demonstrated tetrasomic segregation in cross 80–13 and disomic and/or tetrasomic segregation in 80–11. The unusual segregation ratios observed in cross 80–11 may result from sampling error because of some lethal genetic combination resulting from the parental pairing. This suggestion is supported by the progeny phenotypes observed for a glutamate oxaloacetic transaminase (GOT) locus (A.A.T. E.C. 2.6.1.1). Both parents were asymmetrically heterozygous for this locus. Therefore, the gametic ratios predicted for both models are the same. Progeny phenotypes did not conform to this expectation, however, ($p < 0.001$) in this particular cross, but were observed to conform to Mendelian expectations in cross 80–13 ($p = 0.157$), using the same male parent, and cross 80–14 ($p = 0.920$), using the same female parent (unpublished data). Male 5–008 clearly demonstrated disomic segregation in three of the four crosses involving this individual (80–18, 80–19, and 80–21), and was the most probable type of segregation in the fourth cross (80–20). Male 6–031 demonstrated disomic and/or tetrasomic segregation, with a tetrasomic mode the most probable. Male 6–023 also expressed disomic and/or tetrasomic segregation. In the three crosses involving this male, there was no definite probability as to whether a disomic or tetrasomic mode of segregation was occurring in crosses 80–29 and 80–30, whereas disomy was the most probable in cross 80–27. This may result from sampling error because of the small sample size of tadpoles (45) obtained in this cross.

In crosses involving homozygous females (100^4), only (100^2) gametes will be contributed. Pooling the progeny phenotypes from multiple crosses involving the same male and (100^4) females will, therefore, give a more accurate representation of gametic segregation in the male. These results are shown in Table 6, and suggest that the segregation of gametes in male 1–014 is tetrasomic, but disomic in male 5–008 and disomic and/or tetrasomic in male 6–023.

DISCUSSION

The results reported in this paper suggest that a polymorphism with respect to segregation of gametes exists in *H. versicolor*. This polymorphism presumably

TABLE 5

Progeny phenotypes expected from a tri-allelic (A″A²A′) × *tri-allelic* (A‴A²A′) *cross*

Models*	Female gametes†				Male gametes‡				Progeny phenotypes expected§											
	A″ A	A″ A′	A A	A A′	A‴ A	A‴ A′	A A	A A′	AA AA	AA‴ AA″	A‴A AA	A″A AA	A‴A″ AA′	A‴A AA′	A″A AA′	AA AA′	A‴A A′A′	A″A A′A′	AA′ AA′	A‴A″ A′A′
A	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1
B	1	1	1	1	1	—	—	1	—	1	1	—	1	1	1	1	—	1	1	—
C	1	—	—	1	1	1	1	1	—	1	—	1	1	1	1	1	1	—	1	—
D	1	—	—	1	1	—	—	1	—	1	—	—	—	1	1	—	—	—	1	—
E	2	1	1	2	2	1	1	2	1	4	2	2	4	5	5	4	2	2	4	1
F	1	1	1	1	2	1	1	2	1	2	2	1	3	3	3	3	1	2	2	1
G	1	—	—	1	2	1	1	2	—	2	—	1	1	2	2	1	1	—	2	—
H	2	1	1	2	1	1	1	1	1	2	1	2	3	3	3	3	2	1	2	1
I	2	1	1	2	1	—	—	1	—	2	1	—	1	2	2	1	—	1	2	—

* The models tested were:
A: Disomic segregation in both parents assuming heterozygous bivalents.
B: Disomic segregation in both parents assuming heterozygous bivalents in the female, homozygous bivalents in the male.
C: Disomic segregation in both parents assuming homozygous bivalents in the female, heterozygous bivalents in the male.
D: Disomic segregation in both parents assuming homozygous bivalents.
E: Tetrasomic segregation in both parents.
F: Disomic segregation in the female assuming heterozygous bivalents, tetrasomic segregation in the male.
G: Disomic segregation in the female assuming homozygous bivalents, tetrasomic segregation in the male.
H: Disomic segregation in the male assuming heterozygous bivalents, tetrasomic segregation in the female.
I: Disomic segregation in the male assuming homozygous bivalents, tetrasomic segregation in the female.

† The female phenotype and presumed genotype was A″A²A′.

‡ The male phenotype and presumed genotype was A‴A²A′.

§ Assuming no crossing over between chromatids.

TABLE 6

Observed and expected segregation of MDH-1 *allozymes in combined cross combinations*

Combined parental crosses	Progeny phenotypes: observed (expected with disomic inheritance) /expected with tetrasomic inheritance/				
	AAAA	AAAA'	AAA'A'	χ^2 (df)	Probability
80–11, 80–13	28	131	41		
	(50)	(100)	(50)	20.00(2)	<0.001
	/33.30/	/133.30/	/33.30/	2.27(2)	0.321
80–18, 80–19, 80–21	70	95	67		
	(58)	(116)	(58)	7.15(2)	0.028
	/38.70/	/154.70/	/38.70/	67.14(2)	<0.001
80–27, 80–30	46	131	45		
	(55.50)	(111)	(55.50)	6.69(2)	0.035
	/37/	/148/	/37/	5.31(2)	0.070

results from differences in chromosome pairing during meiosis I. Chromosome associations are not random, however, since this would result in all types of chromosome segregation being expressed in the same individual, producing gametic ratios similar to a tetrasomic mode of segregation. Disomic or tetrasomic inheritance suggests that only bivalents or tetravalents, respectively, are formed. Many crosses do not provide statistical evidence to falsify either model, and suggest that both types of chromosome segregation may occur in some crosses. We believe that this polymorphism is a transitory phase between complete tetrasomy, following genome duplication, and complete disomy.

If homeologous chromosome associations were random at meiosis I, such that there was equal probability of bivalents or tetravalents forming, and preferential pairing during bivalent formation occurred, the segregation ratios would depend upon the type of bivalents formed. If the chromosomes associate equally as heterozygous bivalents and tetravalents, a 1:3:1 ratio of homozygous : heterozygous : alternate homozygous gametes would be expected from a symmetrically heterozygous genotype. If the chromosomes associate as homozygous bivalents however, a 1:10:1 ratio would be expected. There is no current cytological evidence to suggest that such a phenomena could not exist. It is unlikely that chromosome associations producing bivalents and tetravalents would be completely equal. Therefore, the actual disomic-tetrasomic association ratios would be intermediate to the limiting ratios expected with pure tetrasomic and disomic segregation.

The segregation ratios in crosses 80–1, 80–3, 80–10, 80–26, 80–29, 80–30, and 80–31 more closely approximate an intermediate ratio expected with random bivalent and tetravalent formation. Progeny genotypes from crosses 80–10, 80–29, and 80–30 approximate a 1:3:1 ratio and suggest that preferential formation of heterozygous bivalents occurs, while progeny genotypes from crosses 80–26 and 80–31 more closely approximate a 1:11:11:1 and 1:10:1 ratio respectively expected with the preferential formation of homozygous bivalents. The same

male parent was used for crosses 80–26 and 80–31. In crosses involving symmetrically heterozygous males ($A^2A'^2$) and asymmetrically heterozygous females (A^3A'), the A^4:A^3A':$A^2A'^2$:AA'^3 progeny classes will be expected in a 1:4:4:1 ratio and 1:11:11:1 ratio with preferential heterozygous and homozygous bivalent formation respectively. In crosses 80–1 and 80–3 the same male parent was used. The progeny genotypic ratios from cross 80–3 more closely approximate a 1.5:1:1:1.5 ratio for the A^3A':A^4:$A''A^2A'$:$A''A^3$ genotypes respectively, which is expected with equal tetravalent and heterozygous bivalent formation. In cross 80–1 progeny genotypic ratios more closely approximate a 1 : 2.25 : 3 : 3 : 6.5 : 3 : 3 : 2.25 : 1 for A^4 : $A'''A''A^2$: $A'''A^3$+$A''A^3$: $A'''A''AA'$: $A'''A^2A'$+$A''A^2A'$: A^3A' : $A'''AA'^2$+$A''AA'^2$: $A^2A'^2$: $A'''A''A'^2$ genotypes respectively, which is expected with random tetravalent and heterozygous bivalent formation in both parents.

Segregation ratios expected after completely random chromatid crossing over involving double reduction from a quadrivalent formation in symmetrically heterozygous individuals, would produce gametic ratios (2:5:2) (BURNHAM, 1962), that are similar to the 1:3:1 ratio predicted with equal bivalent and tetravalent formation involving the preferential pairing of heterozygous bivalents. Therefore in crosses involving symmetrically heterozygous individuals, it is difficult to falsify either a double reduction or disomic-tetrasomic model unless the sample size is extremely large. In triallelic individuals, however, additional gametic classes are expected with crossing over. For instance, in cross 80–3 involving a male with genotype $A'''A^2A'$, A^2:AA':AA''' : A''' A' : A'''^2:A'^2 gametes in a 5.3 : 6.3 : 6.3 : 3.3 : 1 : 1 ratio respectively, are expected with completely random chromatid crossing over and double reduction. The gametic ratios expected from crossing over within heterozygous bivalents are the same as those predicted from random chromatid segregation, *i.e.*, A^2:AA':$A'''A$:$A'''A'$ in a 1:1:1:1 ratio, respectively. In cross 80–3 with a 100^4 homozygous female, A'''^2A^2 and $A^2A'^2$ genotypes, expected with crossing over, were not observed. Also, in cross 80–1, A^4 : $A''A^3$+$A'''A^3$: A^3A' :$A^2A'''^2$+$A^2A''^2$ + $A'''A''A^2$; $A^2A'^2$: A'''^2AA'+A''^2AA'+$A'''A''AA'$: $A'''AA'^2$+$A''AA'^2$: $A'''A^2A'$+$A''A^2A'$: $A''^2A'^2$+$A'''A''A'^2$ + A'''^2 A'^2 : $A'''A''^2A$+$A''A'''^2A$: AA'^3 : $A'''A''^2A'$+$A'''^2A''A'$: $A'''A'^3$+$A''A'^3$: $A'''^2A''^2$: A'^4 progeny genotypes in a 29 : 70 : 70 : 54 : 54 : 57 : 57 : 122 : 13 : 13 : 13 : 6.5 : 6.5 : 1 : 1 ratio respectively are expected with completely random chromatid crossing over and double reduction in both parents. $A'''A''^2A$, $A''A'''^2A$, AA'^3, $A'''A''^2A'$, $A'''^2A''A'$, $A'''A'^3$, $A''A'^3$, $A'''^2A''^2$ and A'^4 genotypes, expected with crossing over, were not observed. Thus, the evidence obtained from crosses 80–1 and 80–3 suggest that crossing over at the MDH-1 locus is an unlikely explanation for the observed segregation ratios, and that random formation of heterozygous bivalents and quadrivalents is probably involved.

This polymorphism provides an opportunity to study the process of diploidization in a tetraploid species, and to examine the mechanism of this process. Two testable hypotheses can be postulated: (1) one or more regulatory loci determine

chromosome associations and/or chiasmata localizations at meiosis I, and (2) differences in chromosome homology may determine the degree of homeologous associations at meiosis I.

Evidence for the genetic control of chromosome pairing at meiosis I, has been obtained with wheat and ancestral genomes. Formation of bivalents and multivalents in this hexaploid species is controlled by a single gene or small block of genes on the long arm of chromosome 5*B* (RILEY and CHAPMAN 1958; RILEY 1960). These genes were postulated to control premeiotic chromosome pairings. The presence of this chromosome arm prevents multivalent formation at meiosis I. Cells nullisomic for chromosome 5 show higher levels of quadrivalent formation (RILEY and KEMPANNA 1963). Chemically induced mutants have been produced in certain strains that allow chromosome pairing when the chromosome complement is disomic (WALL, RILEY and GALE 1971a). Subsequent placement of these mutants to the long arm of chromosome 5 led to the suggestion that regulatory alleles at a single locus determine the degree of chromosome pairing (WALL, RILEY and GALE 1971a, b).

DRISCOLL, BIELIG and DARVEY (1979) have suggested that multivalent formation in wheat is not determined by premeiotic chromosome pairing, but by the type and number of chiasmata formed between homologs vs. homeologs. Genes controlling chiasma frequency in plants have been reported (REES 1961). There is also evidence for heritable variation with respect to the frequency with which multivalents form in F_2 families of autotetraploid rye (ROSEWEIR and REES 1962). LEWIS (1980) has suggested a combination of both processes; initiation of synapsis through chromosomal associations and distribution of chiasmata in chromosome formations determines the type of configurations observed at meiosis I. Regardless of these alternate explanations, evidence for the genotypic control of chromosome formations during meiosis I is unequivocal in polyploid plants. Similar regulatory loci controlling chromosome associations may also exist in polyploid vertebrates.

Chromosome associations, scored according to quadrivalent formation in a number of cells, in several autotetraploid plant species approximated a binomial distribution (GILLES and RANDOLPH 1951; MORRISON and RAJHATHY 1960; MCCOLLUM 1958; LEWIS 1980). GILLES and RANDOLPH (1951) did, however, note a reduction in quadrivalent formation and consequent increase in bivalent formation through time in autotetraploid maize. MORRISON and RAJHATHY (1960) ascribed the observed chromosome association differences in autotetraploid cereals and grasses they studied to homology, but this explanation is equivocal since they were unable to identify individual homeologous sets. If segregation polymorphisms result from differences in chromosome homology, progeny that arise from a cross between two individuals with both disomic and tetrasomic inheritance would only show disomic-tetrasomic segregation as well, if partial pairing restriction among the homeologous chromosomes exists.

This study provides the first evidence for tetrasomic, disomic, and disomic-tetrasomic inheritance in a vertebrate species. Disomic-tetrasomic segregation would involve the random segregation of quadrivalents and preferentially paired

bivalents. These results are consistent with observations in tetraploid plant species regarding the formation of both bivalents and tetravalents. Residual tetrasomy cannot be invoked to explain the results because the number of individuals observed in each phenotypic class is too large to result from such a process. Residual tetrasomy is characterized by production of unexpected phenotypic classes from crosses involving at least one symmetrically heterozygous parent and is thought to arise from crossover and nondisjunction in homeologous pairings (WRIGHT *et al.* 1980). Linkage of duplicated gene loci, resulting from chromosomal arm fissions and fusions (pseudolinkage), is also a unlikely hypothesis since every *H. versicolor* individual examined to date has an exact doubling of the diploid karyotype. Pseudolinkage in salmonid fish has been used to explain phenotypic ratios approximating a tetrasomic mode of segregation from crosses involving symmetrically heterozygous males (WRIGHT *et al.* 1980). This conclusion may be somewhat premature however, unless it can be shown that segregation of gametes from these multivalents is nonrandom (*i.e.* fusion of homologues with consequent trivalent formation), as opposed to a random tetrasomic segregation. Multivalent formation, consisting primarily of trivalents and quadrivalents, was first reported in *Salvelinus fontinalis* males (DAVISSON, WRIGHT and ATHERTON 1973). Females have shown disomic segregation (MAY, WRIGHT and STONEKING 1979).

These results raise a number of interesting questions: (1) Do unlinked loci fall into blocks of genes that may be controlled by a single regulator (*i.e.*, demonstration of the same inheritance mode for several isozyme loci within an individual, determined to be unlinked in the diploid *H. chrysoscelis*)? (2) Do population differences exist with respect to the mode of inheritance? (3) Do individuals from populations in the suspected transition zone between eastern and western *H. chrysoscelis* show the high degree of disomic segregation expected with an allopolyploid ancestry? Preferential bivalent, compared to random bivalent-tetravalent, formation has been reported in allopolyploid plant hybrids (LEWIS 1980). (4) Do loci assigned to smaller chromosomes in the karyotype show a higher degree of disomic segregation than loci assigned to large chromosomes? JOHN and HENDERSON (1962) have suggested that multivalent formation would be highest in homeologous chromosome pairs having the greatest relative length in the chromosome complement of a species. Therefore, loci located on these chromosomes may be expected to show tetrasomic inheritance in most cross combinations. (5) Have some homeologs completely diploidized? (6) Is there any differences between the sexes with respect to chromosome segregation? Further crosses from different populations should be of value in answering some of these questions.

We thank Dr. BILL BIRKHEAD, JO ELLEN BOGART, Dr. BILL DAVIS, SUSAN and STEVE JOHNSON, MOIRA FERGUSON, DAVID GREEN, BRIAN REILLY, DAVID SERVAGE and CHARLES SULLIVAN for helping to collect the specimens used in this study. Dr. D. M. BRITTON, MOIRA FERGUSON and DAVID GREEN read the manuscript and provided many helpful suggestions. Special thanks are also extended to an anonymous reviewer for constructive criticism of the paper. Technical advice was kindly offered by D. B. RALIN, B. MAY and J. MCGLADE.

LITERATURE CITED

ALLENDORF, F. W., 1975 Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. Ph.D. Thesis, Univ. of Wash. ——, 1978 Protein polymorphism and the rate of loss of duplicate gene expression. Nature **272**: 76–78.

ALLENDORF, F. W. and F. M. UTTER, 1973 Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. Genetics **74**: 647–654. ——, 1976 Gene duplication in the family Salmonidae: III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout (*Salmo clarki*). Hereditas **82**: 15–24.

ALLENDORF, F. W., F. M. UTTER and B. P. MAY, 1975 Gene duplication within the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. pp. 415–432. In: *Isozymes IV*. Edited by C. L. MARKERT. Academic Press, New York.

BACHMANN, K. and J. P. BOGART, 1975 Comparative cytochemical measurements in the diploid-tetraploid species pair of hylid frogs *Hyla chrysoscelis* and *H. versicolor*. Cytogenet. Cell Genet. **15**: 186–194.

BAILEY, G. S., R. T. M. POULTER and P. A. STOCKWELL, 1978 Gene duplication in tetraploid fish: Model for gene silencing at unlinked duplicated loci. Proc. Nat. Acad. Sci. U.S. **75**: 5575–5579.

BAILEY, G. S., A. C. WILSON, J. E. HALVER and C. L. JOHNSON, 1970 Multiple forms of supernatant malate dehydrogenase in salmonid fishes: Biochemical, immunological and genetic studies. J. Biol. Chem. **245**: 5927–5940.

BARRIO, A., 1980 Una nueva species de Ceratophrys (Anura: Ceratophryidae) del dominio chaqueño. Physis (Buenos Aires), Sec. C. **39**: 21–30.

BATISTIC, R. F., M. SOMA, M. L. BEÇAK and W. BEÇAK, 1975 Further studies on polyploid amphibians: A diploid population of *Phyllomedusa burmeisteri*. J. Hered. **66**: 160–162.

BEÇAK, M. L., W. BEÇAK and L. D. VIZOTTO, 1970 A diploid population of the polyploid amphibian *Odontophrynus americanus* and an artificial intraspecific triploid hybrid. Experientia **26**: 545–546.

BEÇAK, W., A. R. SCHWANTES and M. L. SCHWANTES, 1968 Polymorphism of albumin like proteins in the South American tetraploid frog *Odontophrynus americanus* (Salientia: Ceratophrydidae). J. Exp. Zool. **168**: 473–476.

BOGART, J. P., 1967 Chromosomes of the South American amphibian family Ceratophryidae with a reconsideration of the taxonomic status of *Odontophrynus americanus*. Can. J. Gen. Cytol. **9**: 531–542. ——, 1980 Evolutionary implications of polyploidy in amphibians and reptiles. pp. 341–378. In: *Polyploidy*. Edited by W. H. LEWIS. Plenum, New York.

BOGART, J. P. and J. E. BOGART, 1971 Genetic compatibility experiments between some South American anuran amphibians. Herpetologica **27**: 229–235.

BOGART, J. P. and M. TANDY, 1976 Polyploid amphibians: Three more diploid-tetraploid cryptic species of frogs. Science **193**: 334–335.

BOGART, J. P. and A. O. WASSERMAN, 1972 Diploid-tetraploid cryptic species pairs: A possible clue to evolution by polyploidization in anuran amphibians. Cytogentics **11**: 7–24.

BURNHAM, C. R., 1962 *Discussions in Cytogenetics*. Burgess Publ., Minneapolis.

CLAYTON, J. W. and D. N. TRETIAK, 1972 Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Can. **29**: 1169–1172.

DAVISSON, M. T., J. E. WRIGHT and L. M. ATHERTON, 1973 Cytogenetic analysis of pseudolinkage of LDH loci in the teleost genus Salvelinus. Genetics **73**: 645–658.

DRISCOLL, C. J., L. M. BIELIG and N. L. DARVEY, 1979 An analysis of frequencies of chromosome configurations in wheat and wheat hybrids. Genetics **91**: 755–767.

ENGEL, W., J. OP'T HOF and U. WOLF, 1970 Geneduplikation durch polyploide evolution: Die isoenzyme der sorbit dehydrogenase bei herings und lachsartigen fischen (Isospondyli). Humangenetik **9**: 157–163.

ENGEL, W., J. SCHMIDTKE and U. WOLF, 1975 Diploid-tetraploid relationships in teleostean fishes. pp. 449–462. In: *Isozymes IV*. Edited by C. L. MARKERT. Academic Press, New York.

FERRIS, S. D., S. L. PORTNOY and G. S. WHITT, 1979 The roles of speciation and divergence time in the loss of duplicate gene expression. Theor. Pop. Biol. **15**: 114–139.

GERHARDT, H. C., 1974 Mating call differences between eastern and western populations of the treefrog, *Hyla chrysoscelis*. Copeia **1974**: 534–536.

GERHARDT, H. C., S. I. GUTTMAN and A. A. KARLIN, 1980 Natural hybrids between *Hyla cinerea* and *Hyla gratiosa*: Morphology, vocalization and electrophoretic analysis. Copeia **1980**: 577–584.

GILLES, A. and L. F. RANDOLPH, 1951 Reduction of quadrivalent frequency in autotetraploid maize during a period of ten years. Amer. J. Bot. **38**: 12–17.

JOHN, B. and S. A. HENDERSON, 1962 Asynapsis and polyploidy in *Schistocerca paranensis*. Chromosoma (Berl.) **13**: 111–147.

LEWIS, E. J., 1980 Chromosome pairing in tetraploid hybrids between *Lolium perenne* and *L. multiflorum*. Theor. Appl. Genet. **58**: 137–143.

MATHER, K., 1936 Segregation and linkage in autotetraploids. J. Genetics **32**: 287–314.

MAXSON, L., E. PEPPER and R. D. MAXSON, 1977 Immunological resolution of a diploid-tetraploid species complex of tree frogs. Science **197**: 1012–1013.

MAY, B., 1975 Electrophoretic variation in the genus *Oncorhynchus*: The methodology, genetic basis, and practical application to fisheries research and management. M.S. Thesis, Univ. of Wash. University Microfilms #13–10, 112. Ann Arbor, Mich.

MAY, B., J. E. WRIGHT, JR., and M. STONEKING, 1979 Joint segregation of biochemical loci in Salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Bd. Can. **36**: 1114–1128.

MCCOLLUM, G. D., 1958 Comparative studies of chromosome pairing in natural and induced tetraploid *Dactylis*. Chromosoma (Berl.) **9**: 571–605.

MORRISON, J. W. and T. RAJHATHY, 1960 Chromosome behaviour in autotetraploid cereals and grasses. Chromosoma (Berl.) **11**: 297–309.

MECHAM, J. S., 1965 Genetic relationships and reproductive isolation in Southeastern frogs of the genera *Pseudacris* and *Hyla*. Amer. Midland Natur. **74**: 269–308.

RALIN, D. B., 1977 Evolutionary aspects of mating call variation in a diploid-tetraploid species complex of tree frogs (Anura). Evolution **31**: 721–736. ——, 1978 "Resolution" of diploid-tetraploid tree frogs. Science **202**: 335–336.

RALIN, D. B. and R. K. SELANDER, 1979 Evolutionary genetics of diploid-tetraploid species of treefrogs of the genus *Hyla*. Evolution **33**: 595–608.

REES, H., 1961 Genotypic control of chromosome form and behaviour. Bot. Rev. **27**: 288–319.

RILEY, R., 1960 The diploidisation of polyploid wheat. Heredity **15**: 407–429.

RILEY, R. and V. CHAPMAN, 1958 Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature **182**: 713–715.

RILEY, R. and C. KEMPANNA, 1963 The homeologous nature of the non-homologous meiotic pairing in *Triticum aestivum* deficient for chromosome V (5B). Heredity **18**: 287–306.

ROPERS, H. H., W. ENGEL and U. WOLF, 1973 Inheritance of the S-form of NADP dependent isocitrate dehydrogenase polymorphism in rainbow trout. pp. 319–327. In: *Genetics and Mutagenesis of Fish*. Edited by J. H. SCHRODER. Spring-Verlag, Berlin.

ROSEWEIR, J. and H. REES, 1962 Fertility and chromosome pairing in autotetraploid rye. Nature **195**: 203–204.

RUGH, R., 1948 *Experimental Embryology. A Manual of Techniques and Procedures.* Burgess Publ., Minneapolis.

SCHULTZ, R. J., 1980 Polyploidy in fish. pp. 313–340. In: *Polyploidy.* Edited by W. H. LEWIS. Plenum, New York.

STEGEMAN, J. J. and E. GOLDBERG, 1971 Inheritance of hexose-6-phosphate dehydrogenase polymorphism in brook trout. Biochem. Genet. **7:** 279–288.

UTTER, F. M., H. O. HODGINS and F. W. ALLENDORF, 1975 Biochemical genetic studies of fishes: Potentialities and limitations. pp. 213–238. In: *Biochemical and Biophysical Perspectives in Marine Biology* (Vol. 1) Edited by P. C. MALINS and J. R. SARGENT. Academic Press, New York.

UYENO, T. and G. R. SMITH, 1972 Tetraploid origin of the karyotype of catastomid fishes. Science **175:** 644–646.

WALL, A. M., R. RILEY and M. D. GALE, 1971a Wheat mutants permitting homeologous meiotic chromosome pairing. Genet. Res. (Camb.) **18:** 311–328. ——, 1971b The position of a locus on chromosome 5B of *Triticum aestivum* affecting homeologous meiotic pairing. Genet. Res. (Camb.) **18:** 329–339.

WASSERMAN, A. O., 1970 Polyploidy in the common tree toad, *Hyla versicolor* LeConte. Science **16:** 385–386.

WOLF, U., W. ENGEL and J. FAUST, 1970 Zum mechanismus der diploidisierung in der wirbeltierevolution: Koexistenz von tetrasomin und disomen genloci der isocitratdehydrogenase bei der regenbogenforelle, *Salmo irideus.* Humangenetik **9:** 150–156.

WRIGHT, J. E., B. MAY, M. STONEKING and G. M. LEE, 1980 Pseudolinkage of the duplicate loci for supernatant aspartate aminotransferase in brook trout, *Salvelinus fontinalis.* J. Hered. **71:** 223–228.

Corresponding editor: S. WOLFF