FURTHER EVIDENCE FOR A POLYMORPHISM IN GAMETIC SEGREGATION IN THE TETRAPLOID TREEFROG *HYLA VERSICOLOR* USING A GLUTAMATE OXALOACETIC TRANSAMINASE LOCUS

ROY G. DANZMANN[1] and JAMES P. BOGART

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

Manuscript received August 14, 1982
Revised copy accepted December 27, 1982

ABSTRACT

Intra- and interspecific cross combinations between the tetraploid treefrog *Hyla versicolor*, and between *H. versicolor* and the diploid treefrog *Hyla chrysoscelis* were performed. Progeny phenotypes resulting from these crosses were examined electrophoretically using a polymorphic glutamate oxaloacetic transminase (*GOT-1*) locus, to determine the mechanism of chromosome segregation in *H. versicolor*, and to test theoretical expectations for isozyme expression in interspecific (2n × 4n or 4n × 2n) hybrids. In some intraspecific tetraploid crosses progeny phenotypes fit a disomic mode of segregation, whereas in other crosses a tetrasomic mode of segregation was the most probable. Additional crosses produced phenotypic ratios that conformed to either a disomic or tetrasomic mode of segregation. These results suggest that a polymorphism, with respect to segregation of gametes, exists in *H. versicolor*, resulting from differences in chromosome pairings during meiosis I. This polymorphism in gametic segregation occurred in both sexes. Certain crosses, however, produced phenotypic ratios that did not conform to any chromosome segregation model. Progeny phenotypes observed from most interspecific crosses conformed to expected interspecific isozyme staining intensity models. Symmetrical heterozygotes, representing either a single dose for both alternate alleles or double doses for both alternate alleles, were also observed. Such phenotypes are unexpected in triploid progeny. A null allele was postulated to account for the aberrant segregation ratios and phenotypes observed in certain intra- and interspecific crosses.

VARIOUS chromosome segregation modes have been envisioned to occur in tetraploid species. The nature of gametic segregation in tetraploids was first outlined by MULLER (1914), and was based upon a consideration of chromosome segregation from a tetravalent at meiosis I. This mode of segregation is expected in an autotetraploid after tetraploidization and was termed tetrasomic segregation. Later, the process of gametic segregation, assuming the two chromatids from each chromosome acted independently, was outlined by HALDANE (1930), and MATHER (1935, 1936) and was termed random chromatid segregation. MATHER (1935, 1936) postulated that pure tetrasomic (resulting from exclusive

[1] Present address: Department of Zoology, University of Montana, Missoula, Montana 59812.

formation of tetravalents) or disomic segregation (assuming segregation from either homozygous or heterozygous bivalents) of chromosomes may not occur in a tetraploid, and that actual ratios would be intermediate to the limiting ratios expected with either pure tetrasomic or pure disomic segregation.

Bivalent tetrasomic segregation can also result from random formation of homozygous and heterozygous bivalents. Cytological investigations on a number of tetraploid plant species revealed the presence of both bivalents and tetravalents at meiosis I suggesting the mechanical basis for either mode of segregation in these species (LEWIS 1980). Similar observations were made on a pair of recently discovered tetraploid frog species, *Neobatrachus sutor* and *Neobatrachus sudelli* from Australia. Cytological examination of meiosis I in these species revealed both bivalent and tetravalent formation (MAHONY and ROBINSON 1980). Similar meiotic configurations have been found in *Hyla versicolor* (BOGART and WASSERMAN 1972). Such observations suggest that both disomic and tetrasomic segregation may be occurring at a single locus within an individual.

The nature of individual chromosome segregation from homeologous chromosome sets is difficult to ascertain based on cytological examination because individual homeologous sets are not easily identified. The use of isozyme markers in examining chromosome segregation in tetraploids provides a means of assessing the nature of chromosome associations and segregation in tetraploid species. Recently we adopted such an approach, using a polymorphic malate dehydrogenase locus (*MDH-1*), in *H. versicolor* (DANZMANN and BOGART 1982a). Results from this study indicated that a polymorphism exists in *H. versicolor* with respect to the mode of gametic segregation. Certain individuals demonstrated disomic segregation, whereas others demonstrated tetrasomic segregation. A third mode of segregation was also observed, which was intermediate to the limiting types of disomic and tetrasomic segregation, and was postulated to result from the random formation of bivalents and tetravalents. The same mode of segregation was demonstrated by an individual when used in multiple cross combinations suggesting the polymorphism was not simply caused by random bivalent and tetravalent formation. Specific homeologous chromosome associations at meiosis I, resulting in exclusive bivalent or tetravelent formation, were postulated to account for the observed cases of disomic and tetrasomic segregation, respectively. Crosses with segregation ratios intermediate to those expected with pure disomic or tetrasomic segregation were postulated to result from both bivalent and quadrivalent formation of the MDH-bearing chromosomes (DANZMANN and BOGART 1982a).

The purpose of the present study is to further investigate chromosome segregation in the tetraploid treefrog *H. versicolor*, using a polymorphic glutamate oxaloacetic transaminase locus (*GOT-1*) = (*AAT-1*) (EC 2.6.1.1). This enzyme exhibits duplicate gene expression in *H. versicolor* and is dimeric in structure (RALIN and SELANDER 1979). The present study also provides information on segregation of the *GOT-1* bearing chromosomes in both sexes. The previous investigation using MDH was only informative with respect to segregation in the male genome, because the only individuals used with an appro-

priate genotype to distinguish between the alternate models of disomic or tetrasomic segregation were males.

This study also examines *GOT-1* isozyme expression in triploid progeny produced from crosses between diploid *H. chrysoscelis* and tetraploid *H. versicolor*. *H. chrysoscelis* is the diploid progenitor species of tetraploid *H. versicolor* (WASSERMAN 1970). Different electrophoretic staining intensities are expected in triploid progeny as opposed to tetraploid progeny (MAY 1980; DANZMANN and BOGART 1982b). Such theoretical predictions were examined in the interspecific crosses between *H. chrysoscelis* and *H. versicolor*.

MATERIALS AND METHODS

The models describing the alternate modes of disomic and tetrasomic segregation in tetraploids have been outlined previously, and the use of duplicate gene expression in assessing the various modes of chromosome segregation in a tetraploid has been established (ALLENDORF 1975; ALLENDORF, UTTER and MAY 1975). Progeny from intraspecific (4n × 4n) and interspecific crosses (4n × 2n, or 2n × 4n) can theoretically be differentiated by the absence of symmetrically heterozygous progeny (*i.e.*, $A^2A'^2$ or AA') in interspecific crosses. In crosses involving a homozygous diploid genotype (A^2) and a symmetrically heterozygous tetraploid genotype ($A^2A'^2$), A^3:A^2A':AA'^2 progeny genotypes are expected in the same proportions as A^4:A^3A':$A^2A'^2$ progeny genotypes that result from an intraspecific tetraploid cross involving a homozygous (A^4) and symmetrically heterozygous ($A^2A'^2$) tetraploid genotype. When the diploid parent is symmetrically heterozygous (*i.e*, AA') and the tetraploid parent is homozygous (A^4), then only homozygous (A^3) and asymmetrically heterozygous progeny (A^2A') are expected in a 1:1 ratio. Homozygous (A^2) and symmetrically heterozygous (AA') progeny would be expected in equal proportions from an intraspecific diploid cross, where one parent was homozygous (A^2) and the other was symmetrically heterozygous (AA'). The models outlining the various progeny phenotypes and ratios expected from interspecific crosses between *H. versicolor* and *H. chrysoscelis* have previously been reported (DANZMANN AND BOGART 1982b).

The methods of raising the tadpoles and the electrophoretic procedures used have been described (DANZMANN and BOGART 1982a). The only modification in the electrophoretic technique involves the buffer systems used. In addition to an amine citrate, pH 6.5, buffer system (CLAYTON and TRETIAK 1972), a Tris-citrate, pH 6.7, buffer system was also used to score *GOT-1* isozymes (SELANDER *et al.* 1971). *GOT-1* allozyme mobilities were originally designated on an amine citrate, pH 6.5, buffer system. Fragmentation of homodimeric bands was noted on this buffer system, however, and therefore GOT progeny phenotypes were scored on a Tris-citrate, pH 6.7, buffer system, which did not appear to produce this type of artifact. Resolution of isozymes on a Tris-citrate system, however, was not as good as on an amine citrate buffer system.

The sources of the parents used in the various intra- and interspecific crosses, and their respective ploidies are listed in Table 1. The procedures used in making these crosses have been described (DANZMANN and BOGART 1982a).

RESULTS

Intraspecific crosses

Phenotypes observed: Five GOT phenotypes were observed in the progeny from the various intraspecific cross combinations (Figures 1 and 2). Relative electrophoretic staining intensities of GOT suggest duplicate gene expression as the asymmetrical heterozygotes with presumed 3:1 or 1:3 gene dosages approximate a 9:6:1 or 1:6:9 staining intensity, respectively, for a dimeric enzyme (BAILEY *et al.* 1970; ALLENDORF, UTTER and MAY 1975). This is consistent with previous observations on *H. versicolor* GOT expression (RALIN and SELANDER 1979).

TABLE 1

Sources of the two parents used for each cross and their ploidies

Cross No.	Population—[a] Female	Ploidy	Population—Male	Ploidy
79-3	8-4887[b]	2n	10-4870	2n
79-5	8-4887	2n	8-4880	2n
79-6	2-4891	4n	8-4880	2n
79-12	2-4891	4n	10-4877	4n
79-13	2-4891	4n	8-4883	2n
79-14	6-4897	4n	6-4898	4n
79-15	6-4897	4n	6-4899	4n
79-17	2-4928	4n	6-4929	4n
79-18	2-4928	4n	5-4930	4n
79-19	2-4928	4n	5-4931	4n
79-20	1-4932	4n	9-4933	4n
79-21	1-4932	4n	6-4934	4n
79-22	1-4932	4n	9-4935	2n
80-01	1-010[c]	4n	1-011	4n
80-03	1-002	4n	1-011	4n
80-04	1-006	4n	7-007	2n
80-07	7-001	2n	1-014	4n
80-10	4-017	4n	4-005	4n
80-11	1-006	4n	1-014	4n
80-12	1-004	4n	9-001	2n
80-13	1-004	4n	1-014	4n
80-14	1-004	4n	1-030	4n
80-16	1-006	4n	9-001	2n
80-18	3-023	4n	5-008	4n
80-19	5-016	4n	5-008	4n
80-20	1-009	4n	5-008	4n
80-21	1-001	4n	5-008	4n
80-24	1-003	4n	6-005	4n
80-25	6-036	4n	12-001	4n
80-26	1-007	4n	6-031	4n
80-27	1-035	4n	6-023	4n
80-29	1-007	4n	6-023	4n
80-30	1-008	4n	6-023	4n
80-31	1-035	4n	6-031	4n
80-32	1-008	4n	10-003	2n
80-33	1-007	4n	6-017	4n

[a] Parents are listed according to their population of origin and individual catalogue 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′); 7) Hamilton, Harris Co., Georgia (32°49′ 84°52′); 8) Farmington, St. Francois Co., Missouri (37°45′ 90°22′); 9) Lake Riviera, Manitoba (49°40′ 96°34′); 10) McDade, Bastrop Co., Texas (30°17′ 97°15′); 12) Pt. Abino, Ontario (42°55′ 79°05′).

[b] Parents used in 79- crosses are catalogue numbers of JPB.

[c] Parents used in the 80- crosses are catalogue numbers of RGD. These specimens are deposited in the collection of amphibians and reptiles in the Department of Ichthyology and Herpetology at the Royal Ontario Meseum.

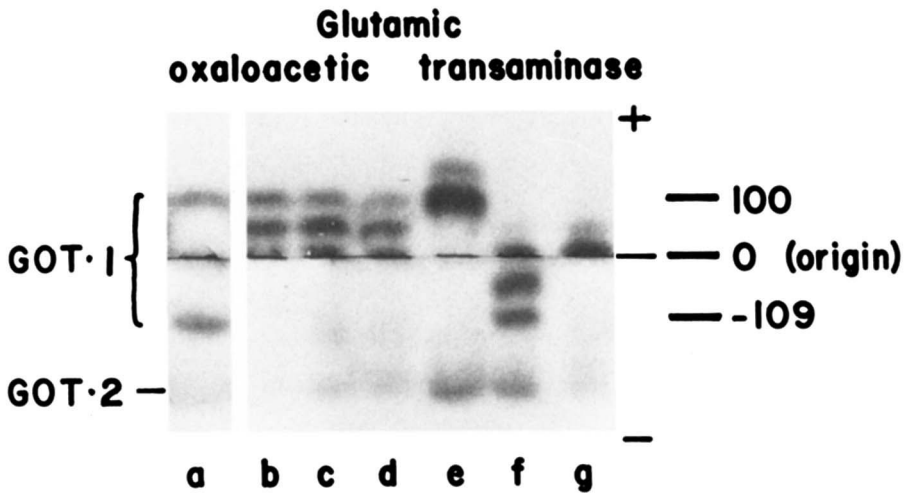

FIGURE 1.—GOT phenotypes obtained in diploid *H. chrysoscelis* using an amine citrate, pH 6.5, buffer system. Homodimeric bands for *GOT-1* are indicated to the right of the gel. *Hyla chrysoscelis* males used for crosses 79-3 and 79-5 had different GOT-1 phenotypes. The same female (4887) was used in both crosses and was homozygous $GOT\text{-}1^{(100^2)}$ (e). Male 4880, $GOT\text{-}1^{(0/-109)}$ (f) was used in cross 79-5. Male 4870, $GOT\text{-}1^{(0^2)}$ (g) was used in cross 79-3. All the tadpoles in 79-3 had a $GOT\text{-}1^{(100/0)}$ (b) phenotype. Tadpoles in 79-5 had $GOT\text{-}1^{(100/0)}$ (c,d) or $GOT\text{-}1^{(100/-109)}$ (a). Fragmentation bands are evidenced by the stained zone above the (100) band (e) and the (0) band (f,g). *GOT-2* is monomorphic.

The allozymes observed were designated *GOT-1* (100) and *GOT-1* (0) according to mobility on an amine citrate buffer system (Figure 1). A third allele *GOT-1* (−109) was encountered in *H. chrysoscelis* from Missouri. *GOT-1* (0) is probably the same allele as *GOT-1* (56) described by RALIN and SELANDER (1979) because this was the most common allozyme encountered in their investigation.

Got-1 (0) was the most common allozyme encountered in this study, and because it had no mobility on an amine citrate buffer system it was necessary to designate the second most common allozyme as (100). The *GOT-1* (100) allozyme of this study may be the same as the *GOT-1* (100) allozyme of RALIN and SELANDER'S study as this was the second most common allozyme that they encountered in *H. versicolor* populations. Two loci for GOT activity were described by RALIN and SELANDER with the most cathodal locus being polymorphic. This was designated *GOT-2*, and presumably corresponds to the *GOT-2* locus described in this study. It is clear that the *GOT-1* locus described in our study is polymorphic, and the *GOT-2* locus is monomorphic. This is the reverse situation of what was described by RALIN and SELANDER. There was no evidence obtained from our study to suggest that there was a more anodal zone of activity to *GOT-1* that would correspond to RALIN and SELANDER'S *GOT-1* locus. Since the buffer system used by RALIN and SELANDER was not indicated it is difficult to relate their isozyme mobilities to our study. Therefore, it was necessary to redesignate the *GOT-1* allozymes according to mobilities on an amine citrate buffer system.

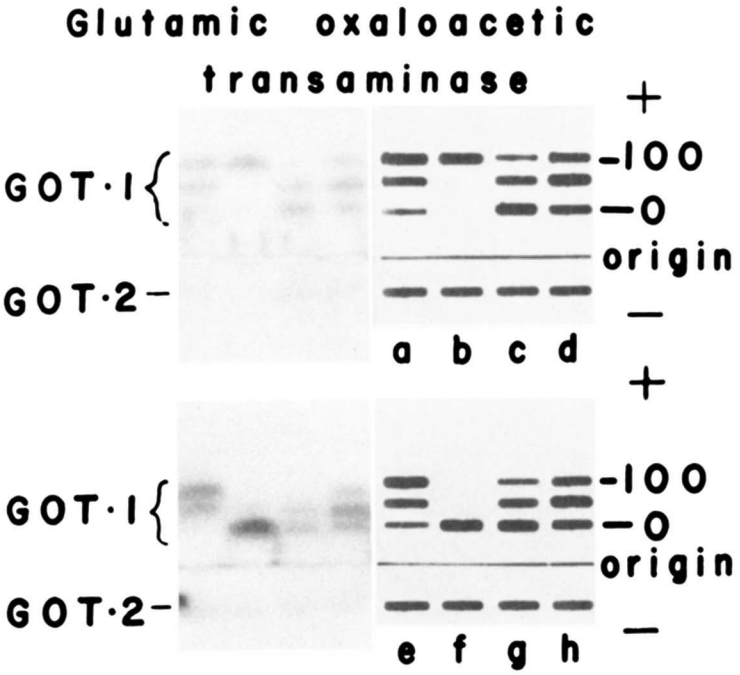

FIGURE 2.—GOT phenotypes obtained in tetraploid *H. versicolor* using a Tris-citrate, pH 6.7, buffer system. Homodimeric bands for *GOT-1* are indicated to the right of the zymogram, and designated according to the mobilities observed on an amine citrate, pH 6.5, buffer system. The presumed genotypes are: *GOT-1*$^{(100^4)}$ (b); *GOT-1*$^{(100^3/0)}$ (a,e); *GOT-1*$^{(100^2/0^2)}$ (d,h); *GOT-1*$^{(100/0^3)}$ (c,g); and *GOT-1*$^{(0^4)}$ (f). *GOT-2* is monomorphic.

Inheritance studies: Segregation in 70 genomes of *H. versicolor* was examined through 25 artificial cross combinations, in which the genotypes of 2116 progeny were scored. From the matings involving at least one symmetrically heterozygous parent, crosses 79-20, 79-21, 80-1, 80-3, 80-10, 80-26, and 80-31 suggest a disomic mode of segregation. Cross 80-30 suggests a tetrasomic mode of segregation, and crosses 79-12, 79-17, 80-18, 80-24 and 80-25 suggest that a mode of segregation intermediate to the limiting ratios of disomic and tetrasomic segregation is occurring (Tables 2 and 3). Only cross 79-20 falsifies the alternate model of tetrasomic segregation at the $P = 0.001$ level. Crosses 79-14, 79-15, 79-18, 79-19, 80-11, 80-13, 80-14, 80-19, 80-20, 80-21, 80-27, 80-29 and 80-33 involved asymmetrically heterozygous parents and could not be used to test for either model. The progeny ratios observed from crosses 79-14, 79-15, 79-18, 79-19, 80-13, 80-14, 80-19, 80-21, 80-27, 80-29 and 80-33 were, however, consistent with Mendelian expectations ($P > 0.10$).

Crosses 80-11 and 80-20, involving an asymmetrically heterozygous female and male parent, did not conform to Mendelian expectations ($P < 0.10$). In cross 80-11 an excess of ($100^3/0$) genotypes and deficiency of ($100^2/0^2$) genotypes was observed. In addition, a single unexpected (0^4) genotype was observed. In cross 80-20 ($100^3/0$) genotypes were observed but not expected. Male 5-008 was used in this cross and in crosses 80-18, 80-19 and 80-21. Segregation ratios were

TABLE 2

Observed and expected segregation of GOT-1 allozymes in intraspecific (4n × 4n) cross combinations

	Parental phenotypes (presumed genotypes)		Progeny phenotypes: observed (expected with disomic segregation) |expected with tetrasomic segregation|						
Cross	Female	Male	100^4	$100^3/0$	$100^2/0^2$	$100/0^3$	0^4	χ^2 (d.f.)	Probability
79-12	$100^2/0^2$	0^4			1	9	5		
					(3.75)	(7.50)	(3.75)	1.63 (2)	0.443
					|2.50|	|10|	|2.50|	2.02 (2)	0.364
79-14	$100/0^3$	$100/0^3$			0	7	1		
					|(2)|	|(4)|	|(2)|	2.81 (2)	0.245
79-15	$100/0^3$	$100/0^3$			1	7	1		
					|(2.25)|	|(4.50)|	|(2.25)|	1.39 (2)	0.499
79-17	$100^3/0$	$100^2/0^2$	0	13	6	2			
			(2.62)	(7.87)	(7.87)	(2.62)		4.68 (3)	0.197
				(10.5)	(10.5)[a]			1.90 (1)	0.168
			|1.75|	|8.75|	|8.75|	|1.75|		3.11 (3)	0.375
79-18	$100^3/0$	0^4			68	65			
					|(66.50)|	|(66.50)|		0.03 (1)	0.862
79-19	$100^3/0$	0^4		1	3	6	1		
				|(0)|	|(5.50)|	|(5.50)|	|(0)|	0.73 (1)	0.393
79-20	$100^2/0^2$	0^4			0	106	5		
					(27.25)	(55.50)	(27.25)	89.64 (2)	<0.001
						(111)[a]		0.18 (0)	—
					|18.50|	|74|	|18.50|	40.05 (2)	<0.001
79-21	$100^2/0^2$	0^4			0	11	1		
					(3)	(6)	(3)	6.21 (2)	0.045
						(12)[a]		0.02 (0)	—
					|2|	|8|	|2|	2.03 (2)	0.362
80-01	$100/0^3$	$100^2/0^2$		35	108	99	31		
				(34.10)	(102.4)	(102.4)	(34.10)	0.54 (3)	0.817
				|22.75|	|113.75|	|113.75|	|22.75|	10.74 (3)	0.013
80-10	$100^2/0^2$	$100^3/0$	9	25	30	9			
			(9.10)	(27.40)	(27.40)	(9.10)		0.33 (3)	0.840
			|6.10|	|30.40|	|30.40|	|6.10|		2.68 (3)	0.423
80-11	$100/0^3$	$100^3/0$		39	29	20	1		
				|(22.25)|	|(44.5)|	|(22.25)|	|(0)|	17.06 (2)	<0.001
80-13	$100/0^3$	$100^3/0$		34	43	29			
				|(26.50)|	|(53)|	|(26.50)|		3.70 (2)	0.157

TABLE 2—*Continued*

Cross	Parental phenotypes (presumed genotypes)		Progeny phenotypes: observed (expected with disomic segregation) |expected with tetrasomic segregation|						
	Female	Male	100^4	$100^3/0$	$100^2/0^2$	$100/0^3$	0^4	χ^2 (d.f.)	Probability
80-14	$100/0^3$	0^4				26 |(26)|	26 |(26)|	0.01 (1)	0.920
80-18	$100^2/0^2$	$100/0^3$		5 (8.25) |5.50|	20 (24.75) |27.50|	33 (24.75) |27.50|	8 (8.25) |5.50|	4.08 (3) 3.42 (3)	0.244 0.318
80-19	0^4	$100/0^3$				44 |(47.5)|	51 |(47.5)|	0.38 (1)	0.538
80-20	$100/0^3$	$100/0^3$		12 |(0)|	31 |(32.5)|	48 |(65)|	39 |(32.5)|	5.33 (2)	0.070
80-21	0^4	$100/0^3$				35 |(35)|	35 |(35)|	0.01 (1)	0.920
80-24	$100/0^3$	$100^2/0^2$		26 (26) |17.3|	92 (78) |86.7|	71 (78) |86.7|	19 (26) |17.3|	4.51 (3) 7.26 (3)	0.205 0.063
80-25	$100/0^3$	$100^2/0^2$		10 (9.40) |6.25|	36 (28.10) |31.25|	24 (28.10) |31.25|	5 (9.10) |6.25|	4.03 (3) 3.82 (3)	0.249 0.271
80-26	$100/0^3$	$100^2/0^2$		14 (9.9) |6.5|	27 (29.6) |32.9|	28 (29.6) |32.9|	10 (9.9) |6.5|	1.51 (3) 10.40 (3)	0.636 0.015
80-27	0^4	$100/0^3$		1 |(0)|		22 |(22.5)|	22 |(22.5)|	0.00 (1)	—
80-29	$100/0^3$	$100/0^3$			32 |(27.5)|	56 |(55)|	22 |(27.5)|	1.49 (2)	0.475
80-30	$100^2/0^2$	$100/0^3$		16 (22.10) |14.75|	84 (66.40) |73.75|	63 (66.40) |73.75|	14 (22.10) |14.75|	8.56 (3) 2.75 (3)	0.035 0.412
80-31	0^4	$100^2/0^2$			8 (7.5) |5|	15 (15) |20|	7 (7.5) |5|	0.02 (2) 2.71 (2)	0.990 0.368
80-33	$100/0^3$	0^4				66 |(47.5)|	52 |(47.5)|	0.39	0.538

[a] Assuming homozygous bivalents.

TABLE 3

Observed and expected segregation of GOT-1 allozymes in cross 80-3 ($100^2/0^2$) × ($100^2/0^2$)

Models[a]	Progeny phenotypes: observed (expected with disomic inheritance) |expected with tetrasomic inheritance| |(expected with disomic and tetrasomic inheritance)|						
	100^4	$100^3/0$	$100^2/0^2$	$100/0^3$	0^4	χ^2 (d.f.)	Probability
	0	52	90	64	1		
A	(12.90)	(51.75)	(77.40)	(51.75)	(12.90)	26.55 (4)	<0.001
B	—	(51.75)	(103.5)	(51.75)	—	4.30 (2)	0.367
C	|5.75|	|46|	|103.5|	|46|	|5.75|	16.88 (4)	0.002
D	|(8.62)|	|(51.75)|	|(86.20)|	|(51.75)|	|(8.62)|	16.32 (4)	0.003
E	—	|(34.50)|	|(138)|	|(34.50)|	—	49.10 (2)	0.001

[a] The models tested are: A) disomic segregation in both parents assuming heterozygous bivalents; B) disomic segregation in both parents assuming heterozygous bivalents in one parent and homozygous bivalents in the other; C) tetrasomic segregation in both parents; D) disomic segregation in one parent with heterozygous bivalents and tetrasomic segregation in the other; and E) disomic segregation in one parent with homozygous bivalents and tetrasomic segregation in the other.

random according to Mendelian expectation for these latter three crosses. It is therefore suggested that the progeny phenotypic ratios are a result of aberrant segregation of gametes in the female. Crossing over involving double reduction would produce (100^2) gametes in this parent.

In cross 79-19 unexpected ($100^3/0$) and (0^4) genotypes were observed (Table 2). In cross 79-17 an excess of ($100^3/0$) genotypes were also observed but not expected. This suggests that the aberrant progeny ratios observed in crosses 79-17 and 79-19 also result from nonrandom segregation in female parent (2-4928), which was used for both these crosses. In cross 79-20 and 79-21 the most probable mode of segregation was disomic, assuming homozygous bivalents in the female parent. Since both males used in these crosses were homozygous (0^4), only ($100/0^3$) progeny genotypes should result. The (0^4) genotypes observed in the progeny from these two crosses are therefore unexpected.

In cross 80-3, involving two symmetrically heterozygous parents, the most probable mode of segregation was disomic in both parents (P = 0.367) (Table 3), under the assumption of homozygous bivalents in one parent and heterozygous bivalents in the other. This is consistent with results from cross 80-1, in which the same male parent (1-011) was used. It is assumed therefore, that the female in cross 80-3 was segregating homozygous bivalents, since a disomic mode with heterozygous bivalents was the most probable in cross 80-1, using male (1-011) (Table 2). The single (0^4) genotype observed in cross 80-3 is unexpected according to model B (Table 3).

Interspecific crosses

Phenotypes observed: The GOT-1 phenotypes observed in the triploid progeny are essentially the same as those observed from tetraploid progeny (Figure 2), except that symmetrically heterozygous progeny are not expected. In asymmetrically heterozygous individuals the only difference between the phenotypes

produced by different ploidy levels is with respect to the intensity of isozyme staining after electrophoresis. Asymmetrically heterozygous tetraploid genotypes (A^3A') are expected to show a 9:6:1 staining intensity for AA:AA':$A'A'$ isozymes, respectively, for a dimeric enzyme. Asymmetrically heterozygous triploid genotype (A^2A') should exhibit a 4:4:1 staining intensity for AA:AA':$A'A'$ isozymes, respectively (BAILEY *et al.* 1970; MAY 1980; DANZMANN and BOGART 1982b). Both genotypes (A^3A' and A^2A'), should result in asymmetrically staining isozyme phenotypes. The observation of such phenotypes in triploid progeny were assumed to represent A^2A' or AA'^2 genotypes. A single electrophoretic band in triploid progeny was assumed to posses the genotype (A^3 or A'^3). Symmetrically heterozygous triploid progeny are unexpected because such phenotypes theoretically represent either a ($A^2A'^2$) or (AA') genotype. These latter phenotypes were observed in certain interspecific crosses however, and their possible origin is discussed in the following section.

Inheritance studies: Segregation in 30 genomes of *H. versicolor* and *H. chrysoscelis* was examined through eight interspecific and two intraspecific diploid cross combinations, in which the genotypes of 461 progeny were scored (Table 4). Crosses involving an asymmetrically heterozygous tetraploid female and homozygous diploid male (80-4, 80-12 and 80-16) produced progeny phenotypes (Table 4) which conformed to a (4n × 2n) interspecific model (Model A, Table 4).

Symmetrically heterozygous progeny were observed in cross 80-12 and 80-16, which were unexpected (Table 4). Both these crosses involved the same male parent (9-001) but different female parents. It is not possible to attribute the origin of the symmetrically heterozygous progeny to crossing over and double reduction in the tetraploid female since this would produce (100^2) gametes, which should result in a (100^2/0) progeny phenotype instead of (100/0). Two possible explanations for the anomalous phenotypes are (1): failure of the female (*0*) allele to be expressed in some (*100/0*) gametic contributions, and (2): failure of the male genotype to be contributed to the resulting zygote, when the female gametic contribution is (*100/0*). This could result from aneuploidy through nondisjunction, gene regulatory differences or structural mutations affecting GOT isozyme expression.

In the single cross (80-7) involving a homozygous diploid female (0^2) and asymmetrically heterozygous tetraploid male (100^3/0) progeny phenotypes clearly did not correspond to a (2n × 4n) interspecific model (Table 4). Both classes of asymmetrically heterozygous progeny are expected in such a cross, in a 1:1 ratio. Only one asymmetrically heterozygous class (*100/0*^{2}) was observed however, with an equal proportion of symmetrical heterozygotes.

In crosses involving a symmetrically heterozygous tetraploid female and homozygous diploid male (79-22 and 80-32), progeny phenotypes corresponded to an interspecific (4n × 2n) model in one cross (80-32), but not the other (79-22). The two symmetrically heterozygous progeny observed in cross 80-32 were unexpected. In cross 79-22 the phenotypes observed did not conform to either an intraspecific diploid or tetraploid model or either interspecific model. The large proportion of symmetrically heterozygous progeny phenotypes observed in this cross are not expected in triploid progeny.

TABLE 4

Observed and expected segregation of GOT-1 *allozymes in inter- and intraspecific diploid cross combinations*

Model[a]	Cross	Parental phenotypes (presumed genotypes)		Progeny phenotypes: observed (expected with disomic inheritance) |expected with tetrasomic inheritance|											
		Female	Male	*A'A'A"A"* or *A'A"*	*AAA* or *AAAA* or *AA*	*AAA'* or *AAAA'*	*AA'A'* or *AA'A'A'*	*A'A'A'* or *A'A'A'A'* or *A'A'*	*AAA"A"* or *AA"*	*AAA'A'* or *AA'*	*AAA"* or *AAAA"*	*AA'A"* or *AAA'A"*	*A'A'A"* or *A'A'A'A"*	χ^2 (d.f.)	Probability
	79-6	*AAA'A'*	*A'A"*	0		3	11	11	0	0	0	6	9		
A						(5)	(10)	(5)			(5)	(10)	(4)	14.25 (5)	0.014
A						|3.33|	|13.33|	|3.33|			|3.33|	|13.33|	|3.33|	29.63 (5)	0.001
B				(10)				(10)	(10)	(10)				27.01 (3)	<0.001
	79-13	*AAA'A'*	*AA'*		0	12	5	5	0						
A					(2.75)	(8.25)	(8.25)	(2.75)						5.15 (3)	0.161
A					|1.83|	|9.15|	|9.15|	|1.83|					6.92 (3)	0.074
B					(5.25)			(5.25)	(10.5)					13.83 (2)	0.001
	79-22	*AAA'A'*	*A'A'*			1	34	2		22					
A						(14.75)	(29.5)	(14.75)		—				22.62 (2)	<0.001
A						|9.83|	|39.33|	|9.83|		—				13.12 (2)	0.001
B								(29.5)		(29.5)				26.37 (1)	<0.001
C							(29.5)	(14.75)		(14.75)[b]				13.80 (2)	0.001
	80-4	*AA'A'A'*	*A'A'*				22	18							
A & C							|(20)|	|(20)|						0.22 (1)	0.635
	80-7	*A'A'*	*AAAA'*				14			11					
A & C						|(12.5)|	|(12.5)|			—				11.60 (1)	<0.001
	80-12	*AA'A'A'*	*A'A'*				44	49		6					
A & C							|(49.5)|	|(49.5)|		—				0.51 (1)	0.477
	80-16	*AA'A'A'*	*A'A'*				32	37		2					
A & C							(35.5)	(35.5)		—				0.28 (1)	0.596
	80-32	*AAA'A'*	*A'A'*			18	54	13		2					
A						(21.75)	(43.5)	(21.75)[b]		—				5.91 (2)	0.052
A						|14.50|	|58|	|14.50|		—				0.90 (2)	0.638
B								(43.50)		(43.50)				59.33 (1)	<0.001
C							(43.5)	(21.75)		(21.75)[b]				22.46 (2)	<0.001
	79-3	*AA*	*A'A'*							6					
B										(6)				—	—
	79-5	*AA*	*A'A"*						2	10					
B									(6)	(6)				4.08 (1)	0.043

GOT-1 allozymes are represented according to the following symbols: *GOT-1 (100) = A; (0) = A'; (−109) = A"*.

[a] The models tested are: A) expected from a 2n × 4n or 4n × 2n cross; B) expected from a 2n × 2n cross; and C) expected from a 4n × 4n cross.

[b] Only the disomic mode of segregation is shown for intraspecific tetraploid crosses.

Crosses involving symmetrically heterozygous tetraploid and diploid parents (79-6 and 79-13) conformed to an interspecific (4n × 2n) model with disomic inheritance, assuming heterozygous bivalents in the female parent as the most probable mode of chromosome segregation. This was consistent with results from cross 79-12 (Table 2), using the same female.

Although a disomic mode of inheritance assuming heterozygous bivalents was the most probable in crosses 79-6 and 79-13, the probability for this model actually operating is low. In fact, (0^3) genotypes were observed to occur twice as frequently as (*100/0/−109*) genotypes in cross 79-6 even though more (*100/0/−109*) genotypes are expected according to either model. (0^3) genotypes were also observed to occur more frequently than (0^2/*-109*) genotypes. This could be explained by the unequal segregation of gametes in the male (*8-4880*), so (*0*) gametes are contributed more often than (*−109*) gametes. This is supported by the progeny ratios observed in intraspecific diploid cross 79-5 (Table 4), using the same male. In addition, there appears to be a greater than expected proportion of (0^2) gametes and deficiency of (100^2) gametes contributed by the female (2-4891), which was used in cross 79-6. This phenomenon was also observed in cross 79-12 using the same female (Table 2), where only one (100^2/0^2) phenotype, and five (0^4) phenotypes were observed.

In the intraspecific diploid crosses only (100/0) progeny phenotypes were observed in cross 79-3, which is expected from combining (100^2) and (0^2) parental genotypes. In cross 79-5 a higher proportion of (100/0) than (100/−109) progeny phenotypes were observed, suggesting unequal segregation of gametes in the male parent used this cross, as previously mentioned for cross 79-6.

The mode of inheritance shown by a symmetrically heterozygous individual when used in multiple cross combinations was identical in four out of the five situations in which this phenomenon could be studied. For instance, male 6-031 was used in crosses 80-26 and 80-31, whereas male (1-011) was used in crosses 80-1 and 80-3, with disomic segregation being the most probably in these four crosses. In cross 80-30 and interspecific cross 80-32, the same female (1-008) was used. The most probable mode of segregation in these two crosses was tetrasomic.

In intraspecific cross 79-12 and interspecific crosses 79-6 and 79-13 the same female parent (2-4891) was used. There was no clear evidence to support either a disomic or tetrasomic mode of segregation in crosses 79-6, 79-12 and 79-13, but a disomic mode of segregation was more probable in all three crosses. In intraspecific crosses 79-20 and 79-21 and interspecific cross 79-22, the same female parent (1-4932) was used. In the intraspecific crosses a disomic mode of segregation assuming homozygous bivalents was the most probable, but segregation phenotypes and ratios in the interspecific cross were not found to conform to either mode of segregation (Table 4).

DISCUSSION

The results obtained from this study are in agreement with previous results obtained from examination of *MDH-1* allozyme segregation in *H. versicolor* (DANZMANN and BOGART 1982a). Both these studies suggest that a polymorphism

with respect to segregation of gametes exists in *H. versicolor*. This polymorphism presumably results from differences in chromosome pairing during meiosis I. In addition to a pure disomic or tetrasomic mode of segregation, which result from exclusive formation of bivalents and tetravalents, respectively, results from certain matings suggested an intermediate mode of segregation, which could result from approximately equal formation of bivalents and tetravalents during meiosis I. Completely random chromatid segregation with crossing over and double reduction from a quadrivalent formation could also produce segregation ratios that are intermediate (i.e., 2:5:2) to pure disomic or tetrasomic ratios (BURNHAM 1962). Therefore, the observed intermediate ratios between disomic and tetrasomic segregation could result from pure quadrivalent segregation with a high frequency of crossing over. It is not possible to distinguish between these models given the phenotypes of the parents involved in the GOT crosses.

Segregation modes are also polymorphic between homeologous chromosome sets within an individual since the segregation modes observed at the *MDH-1* locus (DANZMANN and BOGART 1982a) are not identical to those observed at the GOT-1 locus. For example, cross 80-1 suggests that either a disomic or tetrasomic mode of segregation may be occurring at the *MDH-1* locus in the male used for this cross, whereas a disomic mode appears most probable at the *GOT-1* locus. Similarly, the two males used in crosses 80-10, and 80-24, most probably possessed a tetrasomic and disomic mode of segregation, respectively, at the MDH-1 locus. At the *GOT-1* locus a disomic mode of segregation is most probable for cross 80-10, whereas either mode of chromosome segregation is suggested by the results from cross 80-24. Analysis of joint segregation between *GOT-1* and *MDH-1* allozymes indicate that these two loci are unlinked in *H. versicolor* (DANZMANN 1982). Differences in chromosome segregation modes between homeologous chromosome sets would therefore be predicted, based on the evidence of a polymorphism in gametic segregation at one locus.

Segregation at the *GOT-1* locus demonstrated a number of aberrant segregation ratios as mentioned previously. The occurrence of certain phenotypes could possibly be explained on the basis of crossing over involving double reduction. Double reduction gametes are expected in a low frequency, however, and could not explain the phenotypic ratios observed in certain crosses (e.g., 80-11, 80-13 and 80-20) (Table 2). Furthermore, certain crosses (79-19, 79-20, 79-21 and 80-3) possess phenotypes that are not predicted on the basis of crossing over and double reduction. For instance, in crosses 79-20 and 79-21 (0^4) genotypes occurred that are unexpected according to a model of homozygous bivalent formation occurring in the female used for these two crosses (1-4932). If a small percentage of this female's gametes were produced by a process of quadrivalent formation at meiosis I, then (0^2) and (100^2) gametes could also result, in addition to (*100/0*) gametes. If gamete formation results from a quadrivalent at meiosis I, then it appears to be uni-directional, since only (0^2) gametes are produced, resulting in the production of (0^4) progeny. This is unlikely, unless it is also assumed that a regulatory locus is associated with the (*100*) allele that does not allow self pairing between (*100*) allele-bearing chromosomes.

An alternate possibility to explain these unexpected (0^4) progeny phenotypes is nondisjunction of chromosomes during gamete formation. For instance, the lagging of one chromosome from a segregation pair at meiosis II could result in its loss during subsequent gamete formation. This would then produce either a (*100*) or (*0*) gamete depending upon which chromosome was lost. The subsequent zygotes would either be (0^3) or ($100/0^2$). A (0^3) phenotype would be indistinguishable from a (0^4) phenotype, whereas a ($100/0^2$) phenotype would be very similar to a ($100/0^3$) phenotype, the only difference being in isozyme staining intensity (MAY 1980). Chromosomal nondisjunction in polyploid plants is quite common (LEWIS and JOHN 1963), and it is certainly possible that a similar phenomenon occurs in animals.

Examination of cross 79-22 (Table 4), which also used female (1-4932) reveals an unexpected distribution of phenotypes. The only phenotype expected in this interspecific cross, assuming homozygous bivalent formation in the female, is ($100/0^2$). This was only observed in 57% of the progeny however, with (100/0), ($100^2/0$) and ($0^3 + 0^2$) phenotypes composing the remaining phenotypes. ($100^2/0$) and (0^3) phenotypes could arise from the segregation of (100^2) and (0^2) gametes, respectively, through quadrivalent formation in the female. In addition (*100/0*) progeny could arise through nondisjunction, producing (*100*) gametes in the female. The large proportion of (100/0) progeny phenotypes is unusual because an equal proportion of (*0*) gametes are also expected in the female, according to a nondisjunction model; this should result in equal numbers of (0^2) and (100/0) progeny phenotypes. There may be a mechanism operating in this individual that preferentially results in the production of (*100*) instead of (*0*) gametes through nondisjunction. Such gametes would be undetectable in crosses 79-20 and 79-21 because they would result in the formation of ($100/0^2$) phenotypes, which are nearly identical in electrophoretic staining intensity to the expected ($100/0^3$) genotypes.

The symmetrical phenotypes observed in interspecific crosses 79-22, 80-12, 80-16 and 80-32 may also be the result of unreduced gametes being contributed by the female parent. If the second meiotic division is suppressed after fertilization then a 4n gamete would be produced resulting in a 5n individual. If the first meiotic division produced a heterozygous gamete (*i.e.*, 100/0), then suppression of the second meiotic division would result in a ($100^2/0^2$) gamete. Fertilization by a (*0*) gamete would result in a ($100^2/0^3$) progeny genotype. Given a dimeric structure for GOT, then homodimeric (100) bands: heterodimeric: homodimeric (0) bands would be produced in a 4:12:9 ratio, respectively. Because the heterodimeric band would possess the greatest staining intensity, such phenotypes may be interpreted as symmetrical heterozygotes. It is unlikely, however, that unreduced sperm could account for the high proportion of symmetrical heterozygotes observed in interspecific cross 80-7 (Table 4). The occurrence of such unreduced gametes must also be fairly rare, and probably do not account for the large number of symmetrical heterozygotes observed in cross 79-22.

A regulatory mutation affecting the expression of the GOT-1 allozymes or a structural gene mutation may also be operating to produce the observed effect.

It can be postulated that a regulatory locus affecting the expression of a structural gene locus acts to turn off the synthesis of a particular allozyme, thereby producing a null allele ($\emptyset$).

It is possible that such a null allele polymorphism may be operating in interspecific crosses 79-22 and 80-7, because a large proportion of symmetrically heterozygous progeny were produced in these crosses. These symmetrically heterozygous progeny would therefore have the genotype $100/0/\emptyset$. Similarly, the unexpected $100^3/0$ phenotypes produced in intraspecific cross 80-20 (Table 2) may result from a null allele polymorphism operating in the female parent, as suggested by the results. Unexpected $100^3/0$ progeny phenotypes from cross 80-20 may, in fact, have $100^2/0/\emptyset$ genotypes, producing the observed asymmetry in staining. Such progeny phenotypes could result if the female genotype was $100/0^2/\emptyset$.

Crossing over involving double reduction would produce unexpected (100^2) and ($\emptyset^2$) gametes in the female parent used for cross 80-20, if her genotype were $100/0^2/\emptyset$. If crossing over and chromatid segregation were completely random from a quadrivalent formation, the expected gametic series in the female would be; 100^2:$100/\emptyset$:$100/0$:0^2:$0/\emptyset$:$\emptyset^2$ in a 1:3.33:6.66:5.33:6.66:1 ratio, respectively (BURNHAM 1962). In combination with a ($100/0^3$) genotype in the male in which gametic segregation was random: $100^3/0$:$100^2/0/\emptyset$:$100^2/0^2$:$100/0/\emptyset^2$:$100/0^3$:$100/0^2/\emptyset$:0^4:$0^3/\emptyset$:$0^2/\emptyset^2$ genotypes would be produced in a 1:3.33:6.66:1:12:10:5.33:6.66:1 ratio, respectively. ($100^3/0$) and ($100^2/0/\emptyset$) genotypes would produce similar asymmetrical phenotypes, as would ($100/0^3$) and ($100/0^2/\emptyset$) genotypes, whereas ($100^2/0^2$) and ($100/0/\emptyset^2$) genotypes would produce identical symmetrically staining phenotypes. (0^4), ($0^3/\emptyset$) and ($0^2/\emptyset^2$) genotypes would also produce identical phenotypes. The expected phenotypic ratios would be 4.33:7.66:22:13 for $100^3/0 + 100^2/0/\emptyset$:$100^2/0^2 + 100/0/\emptyset^2$:$100/0^3 + 100/0^2/\emptyset$:$0^4 + 0^3/\emptyset + 0^2/\emptyset^2$ genotypes, respectively.

Additional crosses testing the existence of such putative null alleles will need to be performed. Crosses with F_1 progeny from parents presumed to possess a null allele will be required. In addition, enzyme activity levels and gene dosages should be quantified in individuals thought to possess a null allele. Examination for tissue variability of phenotypes with a putative null allele should also be conducted. Differences in tissue expression would suggest that differential gene regulation is involved in producing the null allele polymorphisms, whereas a uniform phenotype expressed throughout all tissues would indicate a structural null allele.

The existence of null alleles in other tetraploid vertebrates has only been documented in tetraploid fishes. In the family Salmonidae, various null alleles at different enzyme loci have been reported from several species (HECKMAN 1971; WRIGHT, HECKMAN and ATHERTON 1975; BRANDES 1978; HARRIS and BRANDES 1978; MAY, WRIGHT and STONEKING 1979; STONEKING, MAY and WRIGHT 1979; MAY, STONEKING and WRIGHT 1980; MAY 1980; STONEKING, WAGNER and HILDEBRAND 1981; STONEKING, MAY and WRIGHT 1981). Evidence has also been presented for the occurrence of a null allele at an LDH-B locus in a carp (*Cyprinus carpio*) population from Europe (ENGEL, SCHMIDTKE and WOLF 1973).

This polymorphism has also been reported from North American populations of carp (FERRIS and WHITT 1977; DANZMANN and DOWN 1982). The existence of this putative null allele has not been firmly established, however, because results from allozyme segregation studies at this locus are still not available. Inheritance studies on polyploid amphibians have not been performed, so information on the segregation of null alleles in this class does not exist. If the unusual segregation ratios observed in *H. versicolor* are the result of a null allele, this species will provide a unique opportunity to study the process of gene silencing in a recent polyploid.

The authors wish to thank NANCY AUSTIN for her help in rearing of the tadpoles used in this study, and LORI LAPSHINSKI and MARY BESSE for typing the manuscript. Thanks are also extended to Dr. FRED W. ALLENDORF, Dr. PETER E. IHSSEN and MOIRA M. FERGUSON for reading the manuscript, and providing many helpful comments. This research was supported by NSERC (Canada) Grant A9866.

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, University of Washington.

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.

BAILEY, G. S., A. C. WILSON, J. F. 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.

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

BRANDES, W. F., 1978 The taxonomic status of the brook trout *Salvelinus fontinalis* in the Great Smoky Mountains National Park based on electrophoretic data. M. Sc. Thesis, Tennessee Technical University.

BURNHAM, C. R., 1962 *Discussion in Cytogenetics.* Burgess Publishing Co., Minneapolis.

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

DANZMANN, R. G., 1982 An examination of gene expression in the tetraploid tree frog *Hyla versicolor* and in interspecific hybrids with the diploid treefrog *H. chrysoscelis.* M. Sc. Thesis, University of Guelph.

DANZMANN, R. G. and J. P. BOGART, 1982a Evidence for a polymorphism in gametic segregation using a malate dehydrogenase locus in the tetraploid treefrog *Hyla versicolor.* Genetics **100:** 287–306.

DANZMANN, R. G. and J. P. BOGART, 1982b Gene dosage effects on MDH isozyme expression in diploid, triploid, and tetraploid treefrogs of the genus *Hyla.* J. Hered. **73:** 277–280.

DANZMANN, R. G. and N. E. DOWN, 1982 Isozyme expression in F_1 hybrids between carp and goldfish. Biochem. Genet. **20:** 1–15.

ENGEL, W., J. SCHMIDTKE and U. WOLF, 1973 Genetic polymorphism of lactate dehydrogenase isozymes in the carp (*Cyprinus carpio*) apparently due to a "null allele". Biochem. Genet. **8:** 281–289.

FERRIS, S. D. and G. S. WHITT, 1977 The evolution of duplicate gene expression in the carp (*Cyprinus carpio*). Experientia **33:** 1299–1301.

HALDANE, J. B. S., 1930 The theoretical genetics of autopolyploids. J. Genet. **22:** 359–372.

HARRIS, J. W. and W. BRANDES, 1978 The genetic control of isocitrate dehydrogenase isozymes in brook trout, *Salvelinus fontinalis* (Mitchell). Genetics **88:** 538.

HECKMAN, J. R., 1971 Development, immunological and distributional properties of lactate dehydrogenase isozymes in trout. Ph. D. Thesis, Pennsylvania State University.

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

LEWIS, K. R. and B. JOHN, 1963 *Chromosome Marker*. Little, Brown and Co., Boston.

MAHONY, N. J. and E. S. ROBINSON, 1980 Polyploidy in the Australian Leptodactylid frog genus *Neobatrachus*. Chromosome (Berl.) **81:** 199–212.

MATHER, K. 1935 Reductional and equational separation of the chromosomes in bivalents and multivalents. J. Genet. **30:** 53–78.

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

MAY, B., 1980 The salmonid genome: Evolutionary restructuring following a tetraploid event. Ph. D. Thesis, Pennsylvania State University.

MAY, B., M. STONEKING and J. E. WRIGHT, 1980 Joint segregation of biochemical loci in Salmonidae. II. Linkage associations from a hybridized Salvelinus genome (*S. namaycush* × *S. fontinalis*). Genetics **95:** 707–726.

MAY, B., J. E. WRIGHT 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.

MULLER, H. J., 1914 A new mode of segregation in Gregory's tetraploid *Primulas*. Am. Nat. **48:** 508–512.

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

SELANDER, R. K., M. K. SMITH, S. Y. YANG, W. E. JOHNSON and J. B. GENTRY, 1971 Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse. Studies in Genetics VI. University of Texas Publication 7103.

STONEKING, M., B. MAY and J. E. WRIGHT, 1979 Genetic variation, inheritance and quaternary structure of malic enzyme in brook trout (*Salvelinus fontinalis*). Biochem. Genet. **17:** 599–619.

STONEKING, M., B. MAY and J. E. WRIGHT, 1981 Loss of duplicate gene expression in salmonids: Evidence for a null allele polymorphism at the duplicate aspartate aminotransferase loci in brook trout (*Salvelinus fontinalis*). Biochem. Genet. **19:** 1063–1077.

STONEKING, M., D. J. WAGNER and A. C. HILDEBRAND, 1981 Genetic evidence suggesting subspecific differences between northern and southern populations of brook trout (*Salvelinus fontinalis*). Copeia **1981:** 810–819.

WASSERMAN, A. O., 1970 Polyploidy in the common tree toad, *Hyla versicolor* LeConte. Science **16:** 285–286.

WRIGHT, J. E., J. R. HECKMAN and L. M. ATHERTON, 1975 Genetic and developmental analysis of LDH isozymes in trout, pp. 375–401. In: *Isozymes III*. Edited by C. L. Markets, Academic Press, New York.

Corresponding editor: R. E. GANSCHOW