Mode of Origin and Sources of Genotypic Diversity in Triploid Gynogenetic Fish Clones (Poeciliopsis: Poeciliidae)

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Manuscript received July 16, 1991
Accepted for publication November 14,1991

ABSTRACT

Most tributaries of the Río Fuerte in northwestern Mexico contain one or more clones of allotriploid fish of the genus *Poeciliopsis*. We used multilocus allozyme genotypes and mitochondrial DNA (mtDNA) haplotypes to examine several potential modes of origin of these gynogenetic all-female fish. The allozyme studies corroborated earlier morphological work revealing the hybrid constitution of two triploid biotypes, *Poeciliopsis 2 monacha-lucida* and *Poeciliopsis monacha-2 lucida*. Each biotype carries one or two whole genomes from the each of the sexual species *P. monacha* and *P. lucida*. Restriction site analysis of mtDNA revealed that *P. monacha* was the maternal ancestor of five electrophoretically distinguishable triploid clones. Four of five clones were marked by closely related, composite, allozyme/mtDNA genotypes, suggesting they had common origins from an allodiploid clone of the *P. monacha-lucida* biotype. Genotypic analysis revealed that all five clones arose via the "genome addition" pathway. Fertilization of unreduced ova in *P. monacha-lucida* females by sperm from *P. monacha* and *P. lucida* males, respectively, gave rise to both biotypes.

APPROXIMATELY 70 clonally reproducing, vertebrate biotypes have been identified. Essentially all are hybrids between two or more biparentally reproducing sexual species, and the majority (64%) are polyploid (VRIJENHOEK *et al.* 1989). Based on studies with unisexual fishes of the genus *Poeciliopsis* SCHULTZ (1969) first postulated that a relationship exists between hybridization, unisexuality, and polyploidy in vertebrates. He suggested that normal meiotic processes often are disrupted in interspecific hybrids. Occasionally, these hybrids produce unreduced ova (AB) that, upon backcrossing with one of the sexual ancestors (species A or B), lead to new polyploid (allotriploid) biotypes (AAB and ABB). We refer to this pathway for the origin of allotriploid biotypes from allodiploid ancestors as the "genome addition" hypothesis.

Based on cytogenetic studies of meiosis in unisexual Poeciliopsis, CIMINO (1972a) considered a variety of pathways for the origin of allopolyploid vertebrates. For example, under what we will refer to as the "genome duplication" hypothesis, suppression of an equational division in an AB hybrid could produce unreduced AA or BB ova which, if fertilized by species A or B, would produce AAB or ABB offspring. Autopolyploid, AAA and BBB, progeny also would result from this process, but self-sustaining populations of autopolyploid unisexual vertebrates have not been found.

Both the "genome addition" and "genome duplication" hypotheses assume that allotriploids arise from preexisting allodiploid ancestors. The existence of allodiploid relatives to many triploid biotypes lends credence to this assumption. For some triploids, however, self-sustaining allodiploid relatives have not been identified, for example: carp (genus *Carassius*), salamanders (*Ambystoma*), gekkonid lizards (*Hemidactylus* and *Heteronotia*), agamid lizards (*Leiolepis*), and some teiid lizards (*Cnemidophorus*); see VRIJENHOEK *et al.* (1989). Either the allodiploid ancestors were unstable and could not persist longer than one or a few generations, or perhaps they never existed.

CUELLAR (1974, 1977) advanced a third, nonhybrid, pathway, referred to as the "spontaneous origin" hypothesis. He suggested that occasional parthenogenetic reproduction is an inherent property of the sexual progenitors. Thus, species A would be capable of producing unreduced ova (AA) that develop spontaneously. To explain the hybrid genotypes of many parthenogenetic lizards, CUELLAR argued that hybridization might be involved as a secondary process. Occasional fertilization of unreduced AA ova by species B would produce an allotriploid AAB. He criticized the hybrid origin pathways advanced by SCHULTZ and CIMINO, because the putative allodiploid ancestors of triploid Poeciliopsis exhibited an oogenetic process (hybridogenesis, involving premeiotic exclusion of the paternal genome, see below) that bore no cytological resemblance to that of the triploids (gynogenesis, characterized by premeiotic doubling,

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see below), thereby providing no mechanistic link between them.

Modern genetic techniques offer an opportunity to discriminate among these hypothetical pathways. Comprehensive allozyme surveys of unisexual populations and their closest sexual relatives have corroborated that essentially all unisexual vertebrates are interspecific hybrids (DAWLEY 1989). Furthermore, studies of maternally inherited mitochondrial DNA (mtDNA) reveal that hybrid origins typically are asymmetrical, occurring in one direction only (*e.g.*, $A_{♀} \times B_{♂}$ males *vs.* $B_{♀} \times A_{♂}$) (BROWN and WRIGHT 1979; AVISE and VRIJENHOEK 1987; MORITZ *et al.* 1989).

mtDNA analysis has been used to refute the "spontaneous-origins" hypothesis for several parthenogenetic lizards (DENSMORE *et al.* 1989; MORITZ, WRIGHT and BROWN 1989). If parthenogenetic lizards arose spontaneously from sexual ancestors and hybridization was secondarily involved (CUELLAR's "spontaneous origins" hypothesis), the paired-homospecific genomes should derive from the maternal parent, and thus should be coupled with mtDNA from the same species (*i.e.*, AA genomes of AAB should be coupled with mtDNA type-A, and vice versa for ABB). Eight of 10 parthenogenetic *Cnemidophorus* species did not reveal the predicted coupling. Conversely, hybrid origins followed by genome addition or duplication could give rise to either ABB-mtDNA^{A} or ABB-mtDNA^{B}, depending on which genome was duplicated or added.

Assuming hybrid origins, genome addition can be discriminated from genome duplication by the presence or absence of heterozygosity in the homospecific pair of genomes (VRIJENHOEK 1990). For example, genome duplication would result in triploids that are homozygous for all genes carried by the homospecific pair of genomes (A^1A^1B). Alternatively, genome addition will result in heterozygosity for many loci carried by the homospecific pair of genomes (A^1A^2B). Postformational mutations will also contribute to homospecific heterozygosity, so the analysis must be restricted to alleles that are polymorphic in the sexual ancestors. Unequivocal evidence for evolution by genome addition exists in trihybrid unisexuals (ABC) that comprise the genomes of three species, such as *Poeciliopsis monacha-lucida-viriosa* (SCHULTZ 1977) and *Cnemidophorus exsanguis* (GOOD and WRIGHT 1984).

The present study used allozyme and mtDNA analysis to discriminate among possible modes of origin of triploid forms of Poeciliopsis inhabiting the Río Fuerte drainage of northwestern Mexico. Each triploid is a hybrid, containing genomes from two sexually reproducing species, *P. monacha* and *P. lucida*. One triploid biotype, *P. 2 monacha-lucida* (to which we refer as MML), has two *monacha* genomes and one from *lucida*; and the other, *P. monacha-2 lucida* (MLL), has two *lucida* genomes and one from *monacha* (SCHULTZ 1969). Both biotypes reproduce by gynogenesis, a clonal mechanism that faithfully replicates the entire triploid genome across generations (SCHULTZ 1967). CIMINO (1972b) showed that the entire triploid genome is replicated during an endomitotic event prior to meiosis. Pairing of replicated chromosomes followed by normal reductional and equational divisions results in production of nonrecombinant triploid ova. Sperm from a coexisting host species (*P. monacha* for MML and *P. lucida* for MLL) is required to activate embryogenesis of triploid eggs, but paternal genes make no contribution to the all-female offspring.

SCHULTZ (1969) proposed that both triploid biotypes arose via genome addition from the hybridogenetic biotype *P. monacha-lucida* (ML). Hybridogenesis is a hemiclonal form of reproduction; only the haploid M genome is transmitted to developing ova. Apparently, M chromosomes selectively attach to a unipolar spindle during a premeiotic cell division, and L chromosomes are discarded prior to the opportunity for synapsis or recombination (CIMINO 1972a). Normal meiosis ensues with a single equational division producing haploid M ova. Fertilization of these ova by L sperm from *P. lucida* males restores diploidy and the ML genotype. Both maternal and paternal traits are expressed in the allodiploid progeny.

Because of the cytological differences between hybridogenesis and gynogenesis, it might be unwarranted to assume that all triploid forms of Poeciliopsis arose from allodiploid unisexuals (CUELLAR 1977). An additional complication exists because both the MML and MLL biotypes comprise several clones that are distinguishable on the basis of allozyme genotypes, histocompatibility differences, and ecological characteristics (MOORE 1977; VRIJENHOEK 1978; SCHULTZ 1982; SCHENCK and VRIJENHOEK 1989). Different clones of a particular biotype (*e.g.*, MML/I *vs.* MML/II, Roman numerals designate distinguishable electromorph or "E-type" clones) might have had independent origins by entirely different pathways. The present results clearly refute both the "spontaneous origins" and "genome duplication" hypotheses for the evolution of these two biotypes. Only SCHULTZ's (1969) "genome addition" hypothesis is consistent with the combined allozyme and mtDNA data for all five clonal strains examined in this study.

MATERIALS AND METHODS

Comprehensive allozyme surveys have not been reported for allotriploid Poeciliopsis. Allozyme surveys have been in progress for the past 18 years, and include genotypes for several thousand sexual and unisexual Poeciliopsis from the Río Fuerte, and adjacent rivers to the north (Río Mayo) and south (Ríos Sinaloa and Mocorito). Unfortunately, these individuals were preserved in a manner making them un-

TABLE 1

Sources of *P. 2 monacha-lucida* and *P. monacha-2 lucida* and their electrophoretic and mtDNA haplotypes

Locality and biotype	Number of specimens: Wild	Laboratory	E-type[a]	Mt-type[b]
A. Arroyo Cuchujaqui at Parque Cuchujaqui (CU)[c]				
P. monacha-2 lucida		1	I	2
B. Arroyo de Guiricoba at El Cajon (CA)				
P. monacha-2 lucida	2		I	2
P. monacha-2 lucida	1		II	2
P. monacha-2 lucida	2		III	4
C. Arroyo de Jaguari at Rancho El Ranchito (JA)				
P. monacha-2 lucida		1	I	15
P. monacha-2 lucida	3	1	II	2
D. Arroyo San Pedro at San Pedro (SP)				
P. monacha-2 lucida		1	II	2

[a] Refer to Table 2.
[b] Refer to Figure 2.
[c] Site designations (in parentheses), see Figure 1.

suitable for the present mtDNA analysis. Herein, we examine laboratory strains of triploid Poeciliopsis (provided by R. J. SCHULTZ, University of Connecticut, Storrs) and recently collected living specimens for which composite allozyme and mtDNA genotypes could be ascertained (Table 1). The allozyme genotypes observed in this smaller sample of triploids have been verified in the more comprehensive survey (R. C. VRIJENHOEK, unpublished data).

Previous electrophoretic studies based on 25 gene loci revealed two electromorph clones (E-types) of *P. 2 monacha-lucida* in the Río Fuerte, MML/I and MML/II (VRIJENHOEK and LESLIE 1977; VRIJENHOEK 1978). MML/I occurs exclusively in the Arroyo de Jaguari (site JA; Figure 1) and its feeder streams. Only one strain (S68-4 Cx-B) was available for study. MML/II is more widespread, having been found in all tributaries of the Ríos Fuerte and Sinaloa that also contain *P. monacha*. We examined a single MML/II strain (S68-5 Cx-B) and three wild specimens from the same locality (JA). Strains of MML/II from the Guiricoba (CA), Cuchujaqui (CU) and San Pedro (SP) tributaries were not available for analysis. An MML/II E-type also occurs in the Río Sinaloa (site CO), but they have not been captured at this site since 1978 and laboratory strains were not maintained. A related E-type, MML/III, occurred in the Guamúchil tributary of the Río Mayo (VRIJENHOEK and LESLIE 1977), but we have not been successful in capturing it since 1980.

Allozyme surveys have not been reported for the second triploid biotype, *P. monacha-2 lucida*. MLL occurs in the Cuchujaqui, Guiricoba, and San Pedro tributaries of the Río Fuerte but is absent from the Jaguari. MLL also occurs in the Río Sinaloa, but living specimens were not available for study. The Guiricoba tributary of the Río Fuerte currently harbors the most diverse populations of *P. monacha-2 lucida*; five wild specimens were available for mtDNA analyses. Although no wild specimens of MLL were available from the Cuchujaqui or San Pedro tributaries, two laboratory strains, one from each locality (SV73-4 Cy and M61-31 Cy, respectively), were available.

All laboratory strains have previously been verified as triploids by means of chromosome counts [$3n = 72$; see CIMINO (1972a) for methods]. Protein electrophoresis was used to identify triploids in the field collections. Patterns of gene dosage at several loci (*Pgm*, *Ldh-1*, *Pgi-1*, *Mp-1* and *Adh-2*) can be used to differentiate allodiploid from allotriploid biotypes (VRIJENHOEK 1975). Electrophoretic techniques and genotypes for 25 loci were described in earlier publications (VRIJENHOEK, ANGUS and SCHULTZ 1977, 1978). For the present study, a 26th locus was added, *Pep-2* [leucyl-glycyl-glycine peptidase, EC 3.4.11; see MULVEY and VRIJENHOEK (1981) for methods]. *Pep-2* also exhibited gene dosage patterns useful for discriminating allodiploid from allotriploid biotypes.

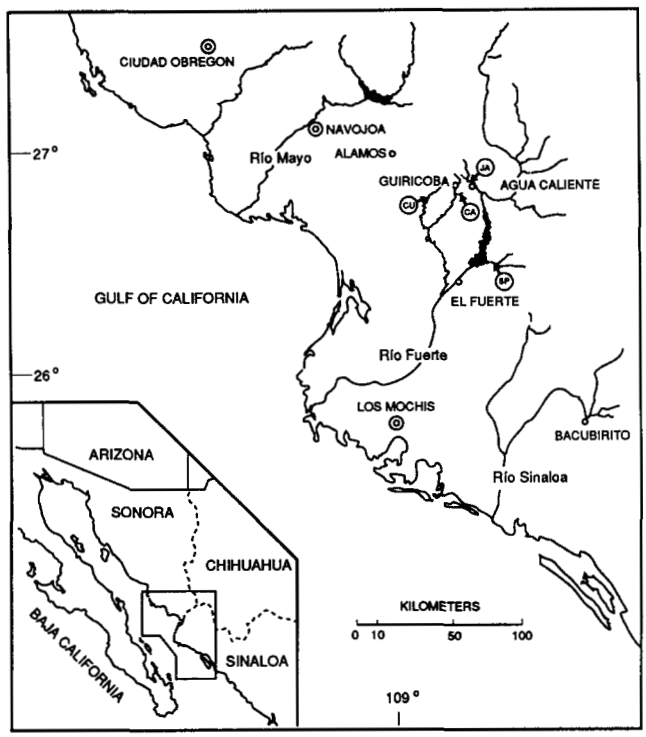

FIGURE 1.—The Ríos Fuerte, Mayo and Sinaloa of northern Mexico and associated tributaries. Sampling localities (arrows) are indicated by site abbreviations: CA, the Arroyo Aguajita de El Cajon; JA, the Arroyo de Jaguari and its feeder streams; SP, Rio San Pedro; CU, Arroyo Cuchujaqui.

mtDNA was isolated from fresh tissues following the methods of LANSMAN *et al.* (1981). Purified mtDNAs were digested according to the manufacturers' specifications with a battery of 17 restriction endonucleases having four-base (*Msp*I), five-base (*Ava*I, *Ava*II, *Hinc*II), and six-base (*Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Nde*I, *Pst*I, *Pvu*II, *Spe*I, *Stu*I, *Xba*I) recognition sequences. Detailed descriptions of laboratory and statistical methods can be found in an earlier publication (QUATTRO, AVISE and VRIJENHOEK 1991).

RESULTS

Allozyme studies based on 25 gene loci identified three E-types of *P. 2 monacha-lucida* (VRIJENHOEK and LESLIE 1977). Two of the E-types, MML/I and MML/II, occurred in the Río Fuerte and one, MML/III, was found only in the Río Mayo. The addition of the *Pep-2* locus identified no new MML E-types in the present sample from the Río Fuerte. Similarly, three E-types of *P. monacha-2 lucida* were found in the Río Fuerte (Table 2). MLL/I occurred at the Cuchujaqui (CU) and Guiricoba (CA) sites, MLL/II occurred at

TABLE 2

Electromorph clones (E-type) of triploid Poeciliopsis, their multilocus genotypes and associated mitochondrial DNA haplotypes

Locus										
Hom.[a]	Het.[b]	*Aat-3*	*Est-4*	*Est-5*	*Idh-2*	*Ldh-1*	*Pep-2*	*Pgm*	E-type	Mt-type(s)
P. 2 monacha-lucida										
aaa	*aa/b*[c]	*bbc*	*bb/a*	*df/d'*[d]	*aaa*	*bb/c*	*aa/c*	*ddd*	I	M.15
aaa	*aa/b*	*bbb*	*bb/a*	*ff/d*	*aa/c*	*bbb*	*aa/b*	*de/d*[d]	II	M.2
P. monacha-2 lucida										
aaa	*a/bb*	*bbb*	*c/aa*	*f/dd*	*a/ac*[d]	*b/cc*	*a/bb*	*e/dd*	I	M.2
aaa	*a/bb*	*bbb*	*b/aa*	*f/dd*	*a/ac*[d]	*bbb*	*bbb*	*e/dd*	II	M.2
aaa	*a/bb*	*bbb*	*c/aa*	*f/dd'*[d]	*a/cc*	*bbb*	*a/bc*[d]	*e/dd*	III	M.4
P. monacha										
a	*a*	*a,b*	*b,c*	*c,d,e,f*	*a,b*	*a,b*	*a,b*	*d,e*		M.1, M.2, M.3, M.4, M.5, M.6, M.7, M.8, M.11, M.14
Poeciliopsis lucida										
a	*b*	*b*	*a*	*d,d'*	*a,c*	*b,c*	*b,c*	*d*		L

[a] Fixed homozygotes for *Adh-1, Gap-1, Ldh-2, Ldh-3, Mdh-1, Mdh-3, Mp-2, Mp-3, Mp-4, Pgd, Sod.*
[b] Fixed heterozygotes for *Aat-1, Aat-2, Adh-2, Gpd, Idh-1, Mdh-2, Mp-1, Pep-1.*
[c] Where diagnostic alleles are known, a slash separates *monacha* and *lucida* alleles.
[d] Heterozygous at homospecific loci.

the Guiricoba (CA) and San Pedro (SP) sites, and MLL/III occurred at Guiricoba (CA).

All MML and MLL triploids were heterozygotes at loci for which the sexual progenitors, *P. monacha* and *P. lucida*, were fixed for alternate alleles (Table 2). An important distinction between the alternative pathways for the evolution of the allotriploid biotypes involves the level of allozyme heterozygosity at the homospecific loci. For *P. 2 monacha-lucida*, both MML/I and MML/II were heterozygous for *P. monacha* alleles at single homospecific MM loci (Table 2). Similarly, for *P. monacha-2 lucida*, MLL/I, MLL/II and MLL/III were heterozygous for common *P. lucida* alleles at one or more homospecific LL loci (Table 2).

Allozyme genotypes at several loci related four of the five triploid E-types. Clones MML/II, MLL/I, MLL/II, MLL/III all carried Pgm^e, a rare *P. monacha* allele that has been found in a single allodiploid clone, ML/XIV (VRIJENHOEK 1984), and a single sexual female in a sample of more than 200 *P. monacha* from the Arroyo de Guiricoba. The same four clones also carry, $Idh\text{-}2^c$, a common allele found in *P. lucida* from the Río Fuerte (SP and CA) and the Río Sinaloa. The exceptional clone, MML/I, contains $Aat\text{-}3^c$, an allele that has yet to be found in any Río Fuerte population of sexual or unisexual Poeciliopsis.

In a previous study of mtDNA restriction site variation in *P. monacha* and *P. monacha-lucida* (QUATTRO, AVISE and VRIJENHOEK 1991), 13 mtDNA haplotypes (Mt-types) were observed among the 44 specimens. For comparative purposes, these haplotypes are listed again in Table 3. All haplotypes found in ML strains were identical or closely related to haplotypes found in natural *P. monacha* populations. Because *P. monacha* and *P. lucida* differ in gel fragment profiles for all 17 restriction endonucleases, maternal ancestry of these ML strains could be assigned unequivocally to *P. monacha*. Similarly, the three mtDNA haplotypes (M.2, M.4 and M.15) found in the allotriploid biotypes MML and MLL could be related to a haplotype in *P. monacha*. A minimum length network interrelating all haplotypes found in Río Fuerte *P. monacha*, *P. monacha-lucida*, and the present sample of triploid strains is illustrated in Figure 2.

The five triploid E-types carried three mtDNA haplotypes (Table 2). Three of the four triploid E-types that shared Pgm^e and $Idh\text{-}2^c$ also contained an identical mtDNA (Mt-type M.2) that was found previously in a Río San Pedro *P. monacha* (QUATTRO, AVISE and VRIJENHOEK 1991) (Figure 2). The mitochondrial genome of the fourth triploid in this group, MLL/III, harbored a unique *Hin*dIII mutation that distinguished it from Mt-type M.2 in the network (Mt-type M.4; Figure 2). The mtDNA genome of E-type MML/I (Mt-type M.15) contained three unique mutations that distinguished it from Mt-type M.2, the most closely related haplotype in the network (Figure 2). No mtDNA divergence was observed within E-type clones inhabiting different tributaries of the Río Fuerte (*e.g.*, MLL/I in the Arroyos Cuchujaqui and Guiricoba, and MLL/II in the Arroyos San Pedro and Guiricoba all carried Mt-type M.2).

DISCUSSION

Joint consideration of allozyme and mtDNA data allow exclusion of all but the "genome addition" hypothesis as the most likely manner by which triploid biotypes of Poeciliopsis arose. First, all five E-type clones were heterozygous for homospecific *monacha*

TABLE 3

Restriction site polymorphism in Poeciliopsis mtDNA, expressed as binary characters (0 = absent, 1 = present)

	Restriction Site																			
		*Ava*II						*Bst*EII		*Eco*RI	*Hin*cII	*Hin*dIII				*Msp*I	*Pst*I	*Xba*I	*Spe*I	*Stu*I
Haplotype	*Ava*I a	b	c	d	e	y	z	f	g	h	i	j	k	l	aa	n	o	p	q	bb
1[a]	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	0	0	0	0
2[a]	1	1	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	0	0
3[a]	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0
4	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	0	0	0
5[a]	1	1	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0
6[a]	1	1	0	0	0	0	0	0	1	0	1	1	1	1	0	0	1	0	0	0
7[a]	1	1	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1	0
8[a]	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0
9[a]	1	1	1	0	0	0	0	0	0	1	0	1	1	0	0	1	0	1	0	0
10[a]	1	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1	0
11[a]	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	1	0
12[a]	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	1	0
13[a]	1	1	0	1	1	0	0	0	0	1	0	1	1	1	0	1	0	0	0	0
14[a]	0	1	0	1	1	0	0	0	0	1	0	1	1	1	0	1	0	0	0	0
15	1	1	0	0	0	1	1	0	0	1	0	1	1	1	0	1	0	0	0	1

[a] From QUATTRO, AVISE and VRIJENHOEK (1991).

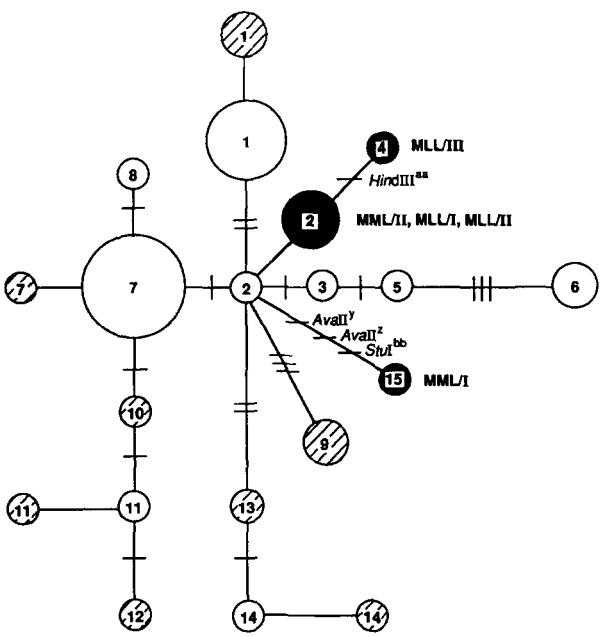

FIGURE 2.—An unrooted minimum length network summarizing relationships among observed mtDNA haplotypes in sexual *P. monacha* (open circles), diploid hybridogenetic *P. monacha-lucida* (crosshatched circles) and triploid Poeciliopsis (blackened circles). For *P monacha*, the sizes of circles are roughly proportional to the frequency of that haplotype. For *P. monacha-lucida* and triploid Poeciliopsis, circles are proportional to the number of different electromorph clones observed with that haplotype. Slashes represent the number of mutations necessary to interrelate haplotypes, and letter codes refer to specific restriction sites polymorphisms. For clarity, the "M" designations preceding haplotype numbers (*i.e.*, M.2) have been dropped. Specific restriction site changes linking haplotypes found in *P. monacha* and *P. monacha-lucida* can be found in a previous publication (QUATTRO, AVISE and VRIJENHOEK 1991).

(MML) or *lucida* (MLL) alleles at one or more allozyme loci, excluding the "genome duplication" hypothesis. Second, two lines of evidence discounted the "spontaneous origin" hypothesis as a viable alternative to genome addition: heterozygosity at homospecific allozyme loci; and the occurrence of *monacha* type mtDNA in MLL. Thus, multiple occurrences of syngamy between haploid sperm from the bisexual species *P. monacha* or *P. lucida* and unreduced ML ova from *P. monacha-lucida* females must have produced all E-type clones of both triploid biotypes, *P. 2 monacha-lucida* and *P. monacha-2 lucida*. These results strengthen the hypothesized relationship between hybridity, unisexuality, and polyploidy in vertebrates (SCHULTZ 1969), and add to a growing body of evidence refuting a role for spontaneous origins of unisexuality (DENSMORE *et al.* 1989; MORITZ, WRIGHT and BROWN 1989; MORITZ *et al.* 1989).

Although multiple genome additions appear to explain the origins of the triploid clones, two lines of evidence indicate that these events were limited to one or a few maternal lineages of *P. monacha-lucida*. First, four of the five triploid E-types examined (MML/II, MLL/I, MLL/II, MLL/III) share polymorphic *P. monacha* and *P. lucida* alleles at several loci. Second, three of the five triploids (MML/II, MLL/I, MLL/II) share a single mtDNA haplotype (Mt-type M.2). The mtDNA haplotypes of the other triploids, MLL/III (Mt-type M.4) and MML/I (Mt-type M.15), differ from Mt-type M.2 by one and three mutations, respectively. This low level of haplotypic diversity contrasts strongly with earlier studies of hybridogenetic lineages of *P. monacha-lucida* from the Río Fuerte. Allozyme and mtDNA diversity in ML essentially matches that found in populations of its maternal progenitor, *P. monacha* (VRIJENHOEK, ANGUS and

SCHULTZ 1978; VRIJENHOEK 1984; QUATTRO, AVISE and VRIJENHOEK 1991).

With few exceptions among vertebrates (*e.g.*, *P. monacha-lucida*), hybridizations leading to successful unisexual lineages appear to be rare events marked by a small fraction of the genetic diversity segregating in the maternal progenitor species (VYAS *et al.* 1990; see reviews in DAWLEY and BOGART 1989). New unisexual lineages can be established if genetic differences between the hybridizing entities are sufficient to disrupt meiotic processes, without adversely affecting viability, fecundity, or other fitness characteristics (MORITZ *et al.* 1989). Given the cytological, developmental, and ecological constraints on unisexuality (VRIJENHOEK 1989), it is not surprising that the origins of triploid Poeciliopsis appear to be restricted to a limited number of hybridizations involving closely related allodiploid progenitors.

These constraints notwithstanding, we cannot be certain that the initial ML hybrids were hybridogenetic; they might have been ephemeral F_1 hybrids, or diploid gynogens. These alternate hypotheses can be evaluated by comparisons of allozyme and mtDNA genotypes in naturally occurring allodiploid lineages of *P. monacha-lucida* from the Río Fuerte. If the triploid biotypes arose from extant lineages of *P. monacha-lucida*, then *monacha* alleles and mtDNA haplotypes marking the triploids would co-occur in diploid hybridogens. Searching for the putative ancestral genotypes among *monacha* genomes of the hybridogens is hampered by allelic diversity in the MML E-types. For example, the original allodiploid lineage leading to MML/I could have had one of four composite genotypes (Table 4). Despite this potentially confounding effect, both MML/I and MML/II share one of the composite genotypes, *bbfabaada*, with hybridogenetic E-type ML/I. However, ML/I strains can be differentiated by two mtDNA haplotypes, M.11 and M.12, neither of which occurs in MML/I or MML/II (Figure 2). Thus, the presently known ML/I composite strains cannot be ancestral to these *P. 2 monacha-lucida* clones. Similarly, allozyme haplotypes found in the other biotype, *P. monacha-2 lucida*, were not observed in the sample of *P. monacha-lucida* hybridogens (Table 4). Perhaps the putative allodiploid ancestor of these triploids flourished at one time, only to be replaced by a more competitive genotype (SCHULTZ 1980). However, given the probable existence of ML hybridogens in unexplored tributaries of the Río Fuerte, as well as in neighboring rivers to the south (Ríos Sinaloa and Mocorito), it is also likely that the putative ancestor remains unsampled.

The triploids might have arisen from F_1 hybrids that were incapable of persisting longer than a single generation. Meiotically unstable hybrids might produce large proportions of unreduced gametes which, after backcrossing to *P. monacha* or *P. lucida*, lead to allotriploid progeny with enhanced fertility or viability. This hypothesis is appealing in the present context, because it might help explain two paradoxes: the absence of extant allodiploid ancestors; and a close genetic relationship among the triploid clones. MORITZ *et al.* (1989) refer to this pathway as the "balance hypothesis" to explain similar observations in some triploid parthenogenetic lizards.

Alternatively, triploid Poeciliopsis might have arisen from gynogenetic diploid intermediates. This hypothesis is appealing for the MML biotype, because *P. monacha-lucida* hybridogens depend on *P. lucida* males for sperm, not *P. monacha* males. If a hybridogenetic female (M^1L) is crossed with a *P. monacha* (M^2) male, diploid M^1M^2 offspring result, which if viable and fertile, have normal meiosis (SCHULTZ 1973a; VRIJENHOEK and SCHULTZ 1974; LESLIE and VRIJENHOEK 1980). However, a gynogenetic allodiploid (M^1L^1) would not produce sexual progeny if mated with a *P. monacha* male. Occasional syngamy between gynogenetically produced M^1L^1 ova and sperm from *P. monacha* (M^2) or *P. lucida* (L^2) would produce the $M^1M^2L^1$ and $M^1L^1L^2$ biotypes via genome addition. Despite the appeal of this hypothesis, gynogenetic diploids of Poeciliopsis have never been found. All naturally occurring allodiploids and all artificially synthesized *P. monacha-lucida* strains reproduce by means of hybridogenesis (SCHULTZ 1969, 1973b; VRIJEN-

TABLE 4

Composite *monacha* haplotypes observed in triploid and diploid unisexual Poeciliopsis

Biotype and E-type	Composite *monacha* haplotype[a]
P. 2 monacha-lucida	
MML/I	*cbdabaada*.15, or *cbfabaada*.15, or *bbdabaada*.15, or *bbfabaada*.15
MML/II	*bbfabaada*.2, or *bbfabaaea*.2
P. monacha-2 lucida	
MLL/I	*bcfabaaea*.2
MLL/II	*bbfababea*.2
MLL/III	*bcfabaaea*.4
P. monacha-lucida	
ML/I	*bbfabaada*.11
ML/I	*bbfabaada*.12
ML/II	*bbcabaada*.13
ML/II	*bbcabaada*.14
ML/III	*abcabaada*.9
ML/IV	*bbdababda*.7
ML/IV	*bbdababda*.9
ML/VII	*bbeaaaadb*.1
ML/VIII	*bbdaacada*.1
ML/XV	*bbfababda*.10

[a] Composite codes consist of observed alleles at the *Aat-3*, *Est-4*, *Est-5*, *Idh-2*, *Ldh-1*, *Pgd*, *Pep-2*, *Pgm* and *Ck-A* loci, respectively, followed by mtDNA haplotype designations (numbers).

HOEK, ANGUS and SCHULTZ 1978; VRIJENHOEK 1984; WETHERINGTON, KOTORA and VRIJENHOEK 1987). However, the present level of sampling as well as data from previous allozyme surveys cannot exclude the hypothesis that a rare gynogenetic diploid occurs or has occurred in the Río Fuerte. Allodiploid gynogens are known in other unisexual fish such as *Poecilia formosa*, *Menidia clarkhubbsi* and *Phoxinus eos-neogaeus*, and they appear to give rise to triploids through genome addition (TURNER *et al.* 1980; ECHELLE *et al.* 1988; GODDARD, DAWLEY and DOWLING 1989).

Although multiple genome additions appear to explain most of the clonal diversity in triploid E-types of Poeciliopsis, other sources of diversity exist. Postformational mutations and recombination between homospecific or heterospecific genomes might contribute to clonal diversity in triploids. Apparently, mutation has contributed to histocompatibility diversity in MML/II. Several strains with this E-type exhibited weak, unidirectional, tissue-graft rejections attributable to postformational, silencing mutations (MOORE 1977). The present electrophoretic data suggests the occurrence of novel mutations in some triploid clones. For example, MML/I carries a unique *Aat-3*^{c} allele that has yet to be found in any population of diploid sexual or unisexual Poeciliopsis. Similarly, MLL/III carries a unique mtDNA haplotype (Mt-type M.4) that can be explained by a single restriction site change from the most common triploid mtDNA haplotype (Mt-type M.2). Since haplotype M.4 has not been found in *P. monacha* or *P. monacha-lucida* (QUATTRO, AVISE and VRIJENHOEK 1991), we cannot determine whether this haplotype is segregating in unsampled populations, has been lost through random lineage extinction, or is a mutant unique to MLL/III. However, the sharing of the *Pgm*^{f} and *Idh-2*^{c} alleles in MLL/I, MLL/II and MLL/III, coupled with the close relationship between Mt-types M.2 and M.4 in these strains, suggests that M.4 is a mutational derivative of M.2, occurring subsequent to the formation of MLL/III.

Although allozyme and mtDNA mutations might have occurred in some triploid lineages of Poeciliopsis, apparent mutational diversity is minimal compared with that of the sexual species *P. monacha* (QUATTRO, AVISE and VRIJENHOEK 1991). Thus, we find no substantive evidence for evolutionary antiquity in these unisexual lineages. The hybridizations and genome additions that gave rise to the triploids must have occurred recently relative to genetic diversification of *P. monacha*. Molecular genetic studies of other unisexual vertebrates also concluded that unisexual populations arose recently (DENSMORE, WRIGHT and BROWN 1989; DENSMORE *et al.* 1989; ECHELLE *et al.* 1989; GODDARD, DAWLEY and DOWLING 1989; MORITZ, WRIGHT and BROWN 1989; MORITZ *et al.* 1989; VYAS *et al.* 1990; QUATTRO, AVISE and VRIJENHOEK 1991). The only exception to this generality involves the diploid hybridogen, *Poeciliopsis monacha-occidentalis* in which a single E-type clone appears to have persisted for hundreds of thousands of clonal generations (QUATTRO, AVISE and VRIJENHOEK 1992).

The unisexual-bisexual complex of poeciliid fishes inhabiting the Río Fuerte offers an unique opportunity to study the delicate balance between the disruption of gametogenesis and the coordination of normal development in hybrid unisexuals. Unlike the hybridogenetic allodiploid *P. monacha-lucida*, the origin of allotriploidy in Poeciliopsis is marked by severe constraints in time and space. The close relatedness of allozyme and mtDNA genotypes in four out of five triploid clones of Poeciliopsis suggests that these clones arose from a single, ancestral, allodiploid lineage. To fully understand the constraints associated with transitions from allodiploidy to allotriploidy, one must identify the allodiploid progenitors and their mode of reproduction. Future studies concerning the origin of these triploid biotypes should expand the geographical survey of potentially ancestral populations of sexual and unisexual Poeciliopsis. We are running out of time for such surveys because many sexual and unisexual populations are now extinct and others are threatened by massive habitat alteration and introductions of exotic species.

We thank P. LEBERG, T. MEAGHER, T. MCGUIRE and P. SMOUSE for helpful comments on the manuscript. We thank R. J. SCHULTZ for providing his laboratory stocks of triploid Poeciliopsis for this study, and for his insightful review of the manuscript. Wild fish specimens were colleced under 412.2.1.3.0 folio 4815 issued jointly by the Departamento de Pesca, by the Secretaria de Desarrollo Urbano y Ecologia, and by the Departamento de Exteriores, Mexico, D.F. Research was supported by National Science Foundation grants BSR88-05360 (J.C.A.) and BSR88-05361 (R.C.V.), the Leathem-Steinetz-Stauber Fund, Rutgers University (J.M.Q.), and the Roosevelt Fund, American Museum of Natural History (J.M.Q.).

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Communicating editor: D. CHARLESWORTH