

ANCIENT VERSUS RETICULATE ORIGIN OF A HEMICLONAL LINEAGE

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Abstract.—Crossing experiments revealed that a diploid hybridogenetic fish (genus *Poeciliopsis*) from the Río Mocorito (Sinaloa, Mexico) is trihybrid. Its haploid maternal genome is inherited clonally (i.e., hemiclonally), and it expresses a mixture of morphological traits found in the closely related species *P. monacha* and *P. viriosa*. Its haploid paternal genome is replaced in each generation by mating with males of a more distantly related sexual species, *P. lucida*. However, expression of mixed (*monacha* × *viriosa*) traits by this hemiclone is also consistent with retention of shared ancestral polymorphisms. If true, this hemiclonal lineage would be one of the few examples of an ancient asexual taxon. We used mitochondrial DNA and allozymes to test whether the maternal progenitor of the Mocorito hybridogen was a recent *P. monacha* × *P. viriosa* hybrid or a remnant of their most recent common ancestor. Our results clearly link the hemiclonal genome to contemporary *P. monacha* and therefore support the hypothesis of a recent origin. Additionally, our findings suggest that this unisexual fish may serve as a vehicle for introgression between two allopatric sexual species.

Key words.—Hybridization, hybridogenesis, introgression, *Poeciliopsis*, unisexual fish.

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Due to an absence of genetic recombination, asexual lineages are generally thought to be evolutionary dead-ends with low rates of adaptive divergence and high rates of extinction (Darlington 1939; Stanley 1975; Williams 1975; Maynard Smith 1978), although a few relatively ancient asexual taxa exist among plants, fungi, and invertebrate animals (Judson and Normark 1996; Mark Welch and Meselson 2000). Nevertheless, no truly ancient asexual taxa are known among the vertebrates (reviewed in Vrijenhoek 1998). A mitochondrial lineage found in a triploid gynogenetic (i.e., sperm-dependent parthenogenetic) salamander of the *Ambystoma jeffersonianum* complex has been isolated from its closest sexual relatives for at least 5 million years (Hedges et al. 1992; Spolsky et al. 1992). Although the mitochondrial lineage associated with this all-female salamander is old, its associated nuclear complement appears to be younger. These all-female salamanders are hybrids that can acquire fresh haploid genomes from their sexual relatives (Bogart and Licht 1986; Bogart 1989). Occasionally, the triploid females produce diploid (reduced) eggs that are truly fertilized by sperm from a sexual relative, generating novel triploid progeny. Similar nuclear genomic replacements occur in triploid forms of the minnow *Phoxinus eos-neogaeus* (Dawley and Goddard 1988), and smaller-scale substitutions of chromosomes or parts of chromosomes are suspected in the topminnow *Poecilia formosa* (Rasch and Balsano 1989; Scharl et al. 1995). Thus, the nuclear genome of these predominantly asexual lineages may be periodically refreshed.

Hybridogenesis, a rare form of clonal inheritance found in a few fish, a frog, and a stick insect (Schultz 1969; Uzzell and Berger 1975; Mantovani and Scali 1992; Carmona et al. 1997) takes nuclear replacement to an extreme. In diploid hybridogens, this hemiclonal (Vrijenhoek et al. 1977) form of inheritance clones the maternal nuclear genome (*M*) and substitutes the paternal nuclear genome (*P*) in each generation

(Fig. 1A). The *MP* hybrid females transmit only a haploid, nonrecombinant *M* genome to their eggs. The *P* genome is excluded during a premeiotic cell division, thereby preventing synapsis, chromosomal assortment, and crossing over (Cimino 1972). Fertilization of haploid *M* eggs by sperm from males of a sexual host species (*P*) restores diploidy in each generation. Because hybridogenetic females require sperm, they must coexist with and potentially compete with females of sexual host species (Vrijenhoek 1989). Nevertheless, the *MP* hybrids obtain several benefits from this unusual form of sexual parasitism (reviewed by Beukeboom and Vrijenhoek 1998). First, the *M* genome is faithfully replicated (cloned) along with whatever special adaptations it encodes and expresses in the diploid state (Vrijenhoek 1979a). Thus, distinct *M* hemiclones specialize ecologically and coexist with one another (Vrijenhoek 1979a, 1984a; Semlitsch et al. 1997). Second, adoption of a new paternal host can gain a hemiclone access to novel environments, for example, increased cold adaptation (Bulger and Schultz 1982). Finally, replacement of the paternal genome in each generation shelters hemiclonal *M* genomes from selection against potentially deleterious recessive mutations that accumulate in nonrecombinant lineages (reviewed in Vrijenhoek 1993).

These benefits may explain the persistence of a hemiclonal lineage of the fish *Poeciliopsis monacha-occidentalis* (hereafter *MO*) that based on mitochondrial evidence may be 150,000 years old (Quattro et al. 1992). This hybridogen, which lives throughout Sonora, Mexico, transmits its *monacha* genome hemiclonally and mates with males of the sexual species *P. occidentalis*. In contrast, an allopatric biotype, *P. monacha-lucida* (*ML*) from southern Sonora and northern Sinaloa (Fig. 2), relies on *P. lucida* males. The *ML* biotype is composed of numerous hemiclonal (*M*) lineages that arose by multiple hybridization events between females of *P. monacha* and males of *P. lucida*, which overlap at several localities in the Ríos Fuerte and Sinaloa (Vrijenhoek 1984b; Quattro et al. 1991). It appears that *P. monacha-lucida* hybridogens in these rivers remain evolutionarily young as old hemiclones are replaced by new ones. Hemiclonal turnover and

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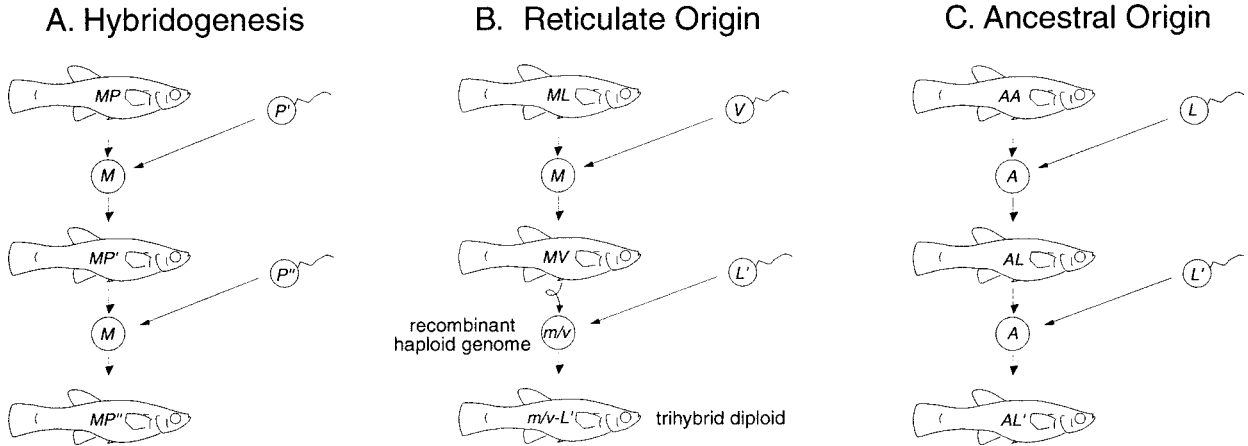


FIG. 1. (A) Hybridogenesis: a diploid hybridogenetic female *MP* (*M* for maternal; *P* for paternal) produces a hemiclinal (haploid) egg *M* with only maternal genes. Haploid sperm *P* (different superscripts represent different males from the same paternal species) fertilizes the *M* egg producing an *MP* female with the same maternal genome as her mother, but different paternal genome. (B) Required steps for the reticulate origin hypothesis: *ML* represents a hybridogenetic female fish (*P. monacha-lucida*) that migrated from another river; *M* represents its hemiclinal (haploid) maternal *monacha* genome; *L* (with different superscripts) represents haploid sperm from different *lucida* males; *V* represents a haploid *viriosa* genome; *m/v* represents recombinant *monacha-viriosa* haploid genome; *m/v-L* represents trihybrid diploid hybridogenetic fish. (C) Ancestral origin hypothesis: *A* represents ancestral lineage; *L* represents *P. lucida*.

opportunities for interclonal selection and adaptive diversification contribute to the ecological breadth and success of the *P. monacha-lucida* biotype, which typically outnumbers the parental species in these rivers (Vrijenhoek 1979a).

South of the Ríos Fuerte and Sinaloa, the Río Mocorito

(Fig. 2) contains a *P. monacha-lucida*-like hybridogen that relies on males of *P. lucida* for sperm. However, *P. monacha* does not live in the Mocorito, so an endemic origin involving *P. monacha* as the maternal parent is unlikely. Furthermore, the Mocorito hybridogen expresses brassy coloration on its

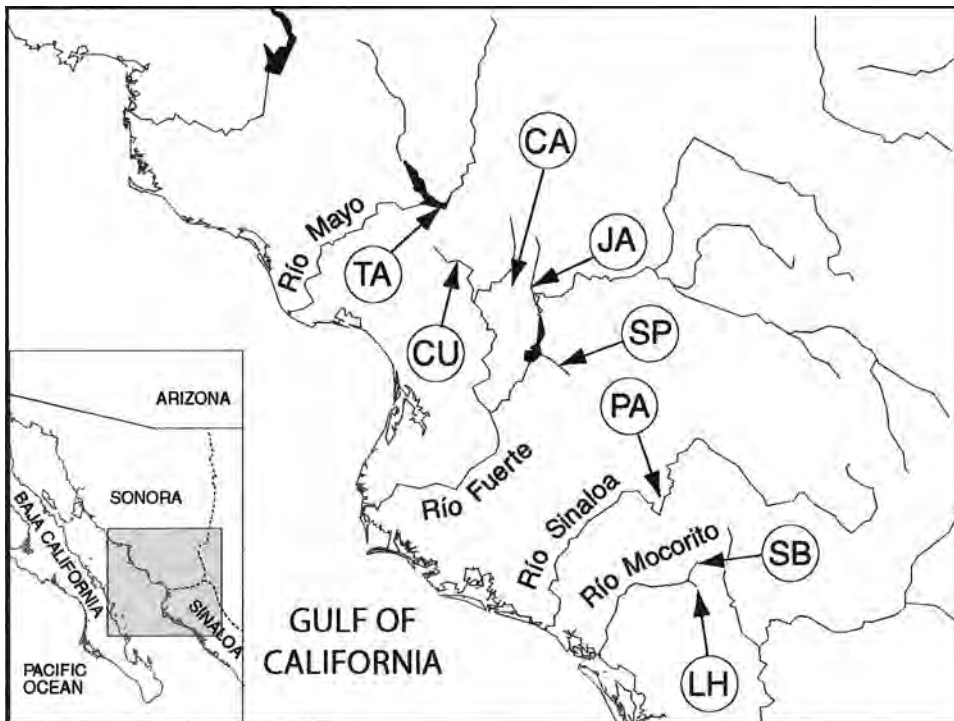


FIG. 2. The southernmost rivers inhabited by hybridogenetic biotypes of *Poeciliopsis*. *Poeciliopsis lucida* and its sperm-dependent types (i.e., *ML* and Mocorito hemiclones) inhabit the Ríos Fuerte, Sinaloa, and Mocorito; *P. monacha* only inhabits the Ríos Mayo, Fuerte, and Sinaloa; *P. viriosa* inhabits the Mocorito and rivers further south. Collection localities are indicated by abbreviations: TA, El Tábelo (27°9.774' N, 108°57.584' W); CU, Cuchujaqui (26°56.4' N, 108°53.4' W); CA, El Cajón (26°52' N, 108°42.8' W); JA, Jaguari (26°54.104' N, 108°40.533' W); SP, San Pedro (26°32.5' N, 108°21' W); PA, El Paso (25°48.65' N, 107°49.9' W); SB, San Benito (25°32.654' N, 107°45.869' W); and LH, La Huerta (25°30.9' N, 107°45.769' W).

body, which typifies *P. viriosa*, a coexisting sexual species. *Poeciliopsis viriosa* and *P. monacha* are morphologically and genetically similar, allopatric sister-species (Miller 1960; Mateos et al. 2002). Vrijenhoek and Schultz (1974) considered two hypotheses concerning the origin of the Mocorito hybridogen: (1) it is a *P. monacha-lucida* (*ML*) lineage that immigrated from the Fuerte or Sinaloa (i.e., migrant origin hypothesis); or (2) it is *P. viriosa-lucida* (*VL*) that originated by hybridization in the R_0 Mocorito (i.e., endemic origin hypothesis). To identify the composition of the unknown maternal genome (*Y*), Vrijenhoek and Schultz (1974) examined morphological traits expressed by offspring from two kinds of crosses: Mocorito hybridogen (*YL*) \times *P. monacha* (*MM*) and Mocorito hybridogen (*YL*) \times *P. viriosa* (*VV*). Progeny from the two crosses (*YM* and *YV*) were compared morphologically to laboratory-reared *P. monacha* (*MM*), *P. viriosa* (*VV*), their reciprocal F_1 hybrids (*MV* and *VM*), and backcross progeny of the hybrids (*MV* \times *MM*, *MV* \times *VV*, etc.). For several traits (brassy coloration, black fin stripe, black tuxedo pattern, dorso-lateral scale counts, preorbital length), the *Y* genome clearly expressed *viriosa* factors, supporting the endemic origin hypothesis, but for other traits (the sizes and number of teeth) the *Y* genome expressed *monacha* factors, supporting the migrant origin hypothesis. These mixed results suggested a third hypothesis, the reticulate origin hypothesis ($Y = m/v$), namely that the *Y* genome of the Mocorito hybridogen contains a mixture of *monacha* and *viriosa* genes.

Indirect support for the reticulate origin hypothesis came from additional crossing experiments (Vrijenhoek and Schultz 1974). Attempts to make *VL* hybrids directly by reciprocal crosses of *P. viriosa* and *P. lucida* produced no offspring, despite use of artificial insemination. In contrast, laboratory crosses of *P. monacha* females \times *P. lucida* males created viable new hybridogenetic *ML* lineages (Schultz 1973). The *viriosa* genome alone appeared to be genetically incompatible with the *lucida* genome. To explain the presence of some *viriosa* traits in the Mocorito hemiclone, Vrijenhoek and Schultz (1974) constructed a multistep scenario (Fig. 1B) that derives from the migrant origin hypothesis: (1) a *P. monacha-lucida* (*ML*) strain migrated from a northern river to the Mocorito, where it was sustained by mating with local males of *P. lucida*; (2) occasional matings between the *ML* strain and males of *P. viriosa* would produce F_1 *monacha* \times *viriosa* (*MV*) hybrids; (3) if the *MV* hybrids had normal meiosis they would produce haploid recombinant (*m/v*) eggs; and (4) if these hybrids mated with males of *P. lucida*, the resulting progeny might be *m/v-L* progeny with hybridogenetic reproduction and a reticulate maternal genome. Steps 2–4 of this scenario were tested and verified with laboratory crosses (Vrijenhoek and Schultz 1974). R_0 Fuerte *ML* females were crossed with *P. viriosa* males. The resulting *MV* progeny had normal recombination. Crosses of *MV* females \times *P. lucida* males produced a single female that founded a new hybridogenetic lineage. Thus, Vrijenhoek and Schultz (1974) concluded that the Mocorito hemiclone was a trihybrid (*P. m/v-L*), beginning as a *P. monacha-lucida* migrant and subsequently incorporating a limited number of *viriosa* genes into its hemiclone genome.

Unfortunately, the molecular markers available in 1974 were not sufficient to test the reticulate origin hypothesis for

the origin of the Mocorito hybridogen. Furthermore, Vrijenhoek and Schultz (1974) did not consider a potentially more parsimonious hypothesis, the ancestral origin hypothesis, that the maternal progenitor of the Mocorito hybridogen was not modern-day *P. monacha* or *P. viriosa*; but that it was their common ancestor (Fig. 1C). Thus, the mixture of traits expressed by this strain could be a result of shared ancestral polymorphisms, rather than reticulation.

Present-day molecular markers can help to distinguish among these hypotheses. To determine the genomic composition and evolutionary age of the Mocorito hybridogen, we examined mitochondrial DNA (mtDNA) sequences and allozymes encoded by 29 gene loci. Six of the allozyme loci are known to differ diagnostically between modern-day *P. monacha* and *P. viriosa*, and their Mendelian inheritance and linkage relationships are known (Leslie 1982; Morizot et al. 1990). Mitochondrial DNA, which is strictly maternally inherited in *Poeciliopsis* (Avisé and Vrijenhoek 1987), has proved to be a powerful tool for assessing the maternal ancestry and evolutionary ages of all-female vertebrates (reviewed by Avisé et al. 1992). Mateos et al. (2002) examined mitochondrial cytochrome *b* and ND2 sequences in 20 named species of *Poeciliopsis* and found that *P. monacha* and *P. viriosa* differ by approximately 10% and 14%, respectively, for the two genes. They estimated conservatively that the two species have been separated for 5–10 million years. If the reticulate origin hypothesis is true, Mocorito hemiclones should have mtDNA consistent with contemporaneous *P. monacha* or *P. viriosa* and a mixture of *monacha* and *viriosa* allozymes. Alternatively, if the ancestral origin hypothesis is true, Mocorito hemiclones should have mtDNA sequences that coalesce with the node connecting *P. monacha* and *P. viriosa* sequences. In this case, some unique (autoapomorphic) allozymes might be expected along with a mixture of *monacha* and *viriosa* allozymes that reflect shared ancestral polymorphisms.

MATERIALS AND METHODS

Collection localities are shown in Figure 2. R_0 Mocorito samples were collected in 1978, 1999, and 2000 from the same two localities (LH and SB) used in previous studies (Schultz 1966; Vrijenhoek and Schultz 1974). Several thousand *Poeciliopsis* were examined morphologically in the field to roughly sort the hybrid biotypes from coexisting sexual species. Externally visible scalation patterns, pigmentation, and mouth shapes were used to tell the sexual species apart (Miller 1960) and distinguish them with reasonable confidence from the all-female hybrids (Vrijenhoek and Schultz 1974; Schultz 1977). Unused fish were returned to their stream with minimal harm. Specimens retained for subsequent genetic analyses were euthanized in ice water (4°C) and frozen immediately on dry ice.

We used cellulose acetate gel electrophoresis (CAGE) to screen specimens for multilocus allozymes (Table 1) that had previously been used to identify different sexual and asexual lineages of *Poeciliopsis* (Vrijenhoek et al. 1978; Morizot et al. 1990). Tissue extracts were prepared as in Vrijenhoek (1979b), and electrophoretic conditions, buffers, and stains followed Hebert and Beaton (1993), unless otherwise noted

TABLE 1. Allozyme loci, optimal tissues, and buffers.

Enzyme	Loci	Tissues ¹	Buffer ²
Phosphoglucose isomerase (Gpi)	<i>Gpi-1, Gpi-2</i>	M, L	TG
6-phosphogluconate dehydrogenase (6Pgdh)	<i>Pgd</i>	M	CAAPM
Glyoxalase-1 (Glo) ³	<i>Glo-1</i>	M	TG
Creatine kinase (Ck) ⁴	<i>Ck-A, Ck-C</i>	M, E	TG
Carboxylesterase (Est)	<i>Est-4, Est-5</i>	M	TG
Guanine deaminase (Gda) ⁵	<i>Gda</i>	L	TG
Alcohol dehydrogenase (Adh)	<i>Adh-2</i>	L	TG
Muscle protein (Ponceau red stain)	<i>MP-1, MP-2, MP-4</i>	M	TG
Phosphoglucomutase (Pgm)	<i>Pgm</i>	M	TG
Isocitrate dehydrogenase (Idh)	<i>Idh-1, Idh-2, Idh-3</i>	M, L, L	TC III
Malate dehydrogenase (Mdh)	<i>Mdh-1, Mdh-2, Mdh-3</i>	E	TC III
Aspartate amino transferase (Aat)	<i>Aat-1, Aat-2, Aat-3</i>	M, E, M	TG
Peptidase (Gly-Leu or Leu-Ala)	<i>Pep-gl or Pep-la</i>	M	TM
Peptidase (Leu-Gly-Gly)	<i>Pep-lgg</i>	M	TM
Adenylate kinase (Ak)	<i>Ak</i>	M	TM
Lactate dehydrogenase (Ldh)	<i>Ldh-1, Ldh-2, Ldh-3</i>	E	TG

¹ M, muscle; L, liver; E, eye.

² TG, tris glycine, pH = 8.5 diluted 1:9; CAAPM, citric acid-amino propylmorpholine, pH = 6.2, diluted 1:19 (Hebert and Beaton 1993); TC III, Tris-citrate buffer III, pH = 7.0, diluted 1:19 (Murphy et al. 1996); TM, 0.5M Tris adjusted to pH 7.4 with maleic acid, undiluted (Richardson et al. 1986).

³ Stain: 2 ml phosphate buffer (2.76 g NaH₂PO₄ in 100 ml water adjusted with to pH = 7.0), four drops reduced glutathione (12.5 mg/ml), 25 µl methylglyoxal, and five drops MTT (D. Morizot, pers. comm.).

⁴ Modified from adenylate kinase recipe (Hebert and Beaton 1993) by adding 30 mg phosphocreatine.

⁵ Stain: 2 ml Tris-HCl pH = 8.0 (Hebert and Beaton 1993), two drops guanine (3.125 mg/ml), ~ 1 unit xanthine oxidase, five drops MTT, five drops PMS, agar overlay (D. Morizot, pers. comm.).

(Table 1). For *P. monacha*, which does not live in the Mocerito, we relied on published data (Vrijenhoek 1979b; Morizot et al. 1990) and analyses of laboratory strains from the Rø Fuerte. Allelic identifications were cross-referenced (side by side) on CAGE membranes with genetically defined laboratory strains (S68–4 Cw and T70–3 Cw) of *P. monacha-lucida* from the Rø Fuerte and collected in 1968 (R. J. Schultz) and 1970 (R. E. Thibault).

To infer maternal ancestry of Mocerito hemiclones, we examined DNA sequences of whole mitochondrial ND2 (1047 bp) and cytochrome *b* (1140 bp) genes in hybridogens previously identified by allozymes. They were compared with new sequences of other wild-caught or laboratory strains of *P. monacha-lucida* (ML), *P. monacha-occidentalis* (MO), and published sequences (Mateos et al. 2002) of sexual *P. monacha* (GenBank accession numbers: AF412173 and AF412131) and *P. viriosa* from Rø Mocerito (AF412133 and AF412175) and San Pedro Lagunillas (a southern locality in the state of Nayarit; AF412132 and AF412133). *Poeciliopsis lucida* (AF412184 and AF412175) was used to root the tree because it is well outside the *monacha-viriosa* clade (Mateos et al. 2002). DNA extraction, amplification, and sequencing methods are described in Mateos et al. (2002). To exclude polymerase error as a source of haplotypic variation, we repeated polymerase chain reaction and sequencing of individuals bearing unique haplotypes.

We used PAUP* 4.0b8 (Swofford 1998) to infer phylogenetic relationships among OTUs. To increase our power of discrimination among *monacha* genomes, we combined the information from the two genes into a single haplotype. We used heuristic searches (with TBR branch swapping and 50 random addition replicates) under maximum-parsimony and minimum-evolution criteria. Minimum-evolution analyses assumed Kimura two-parameter (Kimura 1980) cor-

rected distances. Support for particular nodes was evaluated with bootstrap analyses (1000 heuristic replicates).

RESULTS

Preliminary morphological identifications in the field revealed that populations of *P. lucida*, *P. viriosa*, and related all-female hybrids in the Rø Mocerito were rare compared to other species of *Poeciliopsis* (*P. prolifica*, *P. presidionis*, and *P. latidens*). The Mocerito hybridogens sampled in 1978, 1999, and 2000 all expressed the distinct brassy coloration of *P. viriosa*. Distinction of hybridogens from *P. viriosa* required allozyme analyses.

We examined 29 allozyme loci to assess the nuclear genomic composition of Rø Mocerito *P. monacha-lucida*-like hybridogens (Table 2). Among several hundred fish examined from samples taken in 1978, 1999, and 2000, we identified only 36 specimens of this biotype (20 from SB-1978, 10 from LH-1999, and six from LH-2000). All 36 individuals were fixed heterozygotes for alleles at 14 loci that differed between *P. monacha* and *P. lucida*. They were fixed homozygotes for alleles at 14 loci that were shared between the putative parental species. The hybridogens were polymorphic at one locus (*Ldh-1*) that segregated in local *P. lucida* populations. This polymorphism in the hybridogens was due to substitution of paternal alleles from *P. lucida* (see Vrijenhoek et al. 1977).

We found no evidence for *viriosa* allozymes in the Río Mocerito hybridogen. Alleles at six loci were diagnostic between *P. viriosa* and *P. monacha* (boldface capital letters in Table 2), and none of these alleles existed in the sample of 36 hybridogens from the two Mocerito localities. However, we cannot exclude the possibility that *viriosa* alleles exist in these hybridogens because we identified only a small number

TABLE 2. Allozyme markers at 29 loci in the putative parental species, R0 Mocerito hybridogenetic females, and in a wild-caught *monacha* × *viriosa* male hybrid.

Locus	Putative parental alleles			Hybrid genotypes ¹	
	<i>monacha</i> ²	<i>viriosa</i> ^{3,4}	<i>lucida</i> ³	Hybrid-ogens	Hybrid male
Heterozygous in Mocerito hybridogens					
<i>Aat-1</i>	a	a	b	a b	a a
<i>Aat-2</i>	a	a	b	a b	a a
<i>Adh-2</i>	a	C	b	a b	a C
<i>Est-4</i>	b, c	b	a	b a	b b
<i>Est-5</i>	c, d, e, f	e	d	c d	c e
<i>Glo-1</i>	a	B	b	a b	a B
<i>Gpi-1</i>	a	B	c	a c	a B
<i>Gpi-2</i>	b	b	a	b a	b b
<i>Idh-1</i>	a, b	a	b	a b	a a
<i>Idh-3</i>	b	b	a	b a	b b
<i>Mdh-2</i>	a	a	b	a b	a a
<i>Mp-1</i>	a	a	b	a b	a a
<i>Pep-la</i> or <i>Pep-gl</i>	a	a	b	a b	a a
<i>Pep-igg</i>	a, b	b	c	a c	a b
Homozygous in Mocerito hybridogens					
<i>Aat-3</i>	b	b	b	b b	b b
<i>Ak</i>	a	a	a	a a	a a
<i>Ck-A</i>	a, b	a	a	a a	a a
<i>Ck-C</i>	b	A	b	b b	b A
<i>Gda</i>	b	A	b	b b	b A
<i>Idh-2</i>	a, b	a	a	a a	a a
<i>Ldh-2</i>	a	a	a	a a	a a
<i>Ldh-3</i>	a	a	a	a a	a a
<i>Mdh-1</i>	a	a	a	a a	a a
<i>Mdh-3</i>	a	a	a	a a	a a
<i>Mp-2</i>	a	a	a	a a	a a
<i>Mp-4</i>	a	a	a	a a	a a
<i>Pgd</i>	a, c	B	a	a a	a B
<i>Pgm</i>	d, e	d, F	d	d d	d d
Polymorphic locus in Mocerito hybridogens					
<i>Ldh-1</i>	a, b	b	b, d	b b, b d	b b

¹ Inferred hemiclonal allele in italics.

² From *monacha* genomes found in sexual *Poeciliopsis monacha* populations and hemiclonal genomes in *P. monacha-lucida* strains from the R0 Fuerte and Sinaloa (from Vrijenhoek 1979b, 1984b; Morizot et al. 1990).

³ From R0 Mocerito *P. viriosa* and *P. lucida* populations.

⁴ Boldface capital letter alleles distinguish *P. viriosa* from *P. monacha*.

of diagnostic allozyme markers. It should be noted that *P. monacha* and *P. viriosa* share electromorphs at most (23/29) of the loci examined in this study.

By identifying paternal (*lucida*) alleles in hybridogens, it is possible to infer the multilocus haploid genotypes of their hemiclonal (*M*) genomes (Vrijenhoek et al. 1977, 1978). A single *M* hemiclone was observed among the 36 specimens examined in this study. Interestingly, the *M* genotype found in the Mocerito was not different, based on allozyme markers, from R0 Fuerte *P. monacha-lucida* hemiclone II (see Vrijenhoek et al. 1977).

Mitochondrial haplotypes helped to clarify the origin of the Mocerito hybridogen. We identified seven new ND2 sequences (1047 bp) and seven new cytochrome *b* sequences (~ 1140 bp) that combined into eight composite haplotypes (deposited separately under GenBank accession numbers AF458362–AF458369 for ND2 and AF458370–AF458377 for cytochrome *b*). Separately, each gene region provided limited discrimination among *monacha* genomes, although

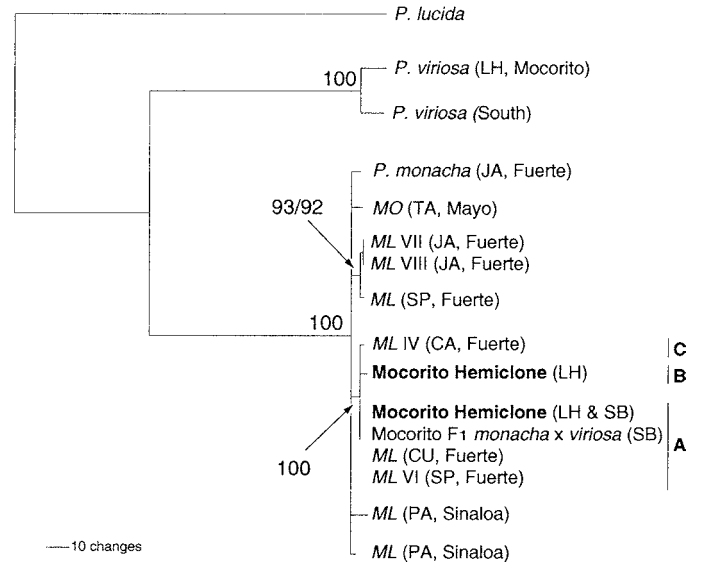


FIG. 3. A strict consensus of most parsimonious trees, based on complete ND2 and cytochrome *b* sequences, depicting relationships among haplotypes of *Poeciliopsis* asexual hybrids *P. monacha-lucida* (*ML*) and *P. monacha-occidentalis* (*MO*) and sexual lineages of *P. monacha* and *P. viriosa*. The tree was rooted with *P. lucida*. Bootstrap support values (>60%) are shown next to nodes (parsimony/distance). A, B, and C correspond to closely related haplotypes of Mocerito and other hemiclones. Localities (in parentheses) are marked in Figure 2.

each gene was clearly divergent among the putative parental species, *P. monacha*, *P. viriosa*, and *P. lucida* (Mateos et al. 2002). Results from maximum-parsimony (Fig. 3) and minimum-evolution (not shown) analyses were broadly congruent. Minor discrepancies between these methods were not supported by bootstrap values above 60%. Both analyses unequivocally grouped Mocerito hybridogens with *P. monacha-lucida* and *P. monacha-occidentalis* hybridogens and *P. monacha* from the R0s Fuerte, Sinaloa, and Mayo. *Poeciliopsis viriosa* haplotypes from the R0 Mocerito and from a southern locality outside the range of the all-female hybrid forms were clearly excluded from the *monacha* group. Two mtDNA haplotypes were found among the 16 Mocerito hybridogens examined in this study (haplotypes A and B; Fig. 3). Fifteen Mocerito hybridogens had haplotype A, which was identical with mitochondrial haplotypes found in R0 Fuerte *P. monacha-lucida* hybridogens from the CU locality and a laboratory strain (hemiclone VI) from the SP locality. Haplotype B, found in a single Mocerito individual, differed from haplotype A by two unique substitutions. A closely related haplotype (C), was found in a R0 Fuerte *P. monacha-lucida* strain, hemiclone IV. Haplotypes A, B, and C together formed a well-supported clade to the exclusion of *P. monacha-occidentalis* from the R0 Mayo (TA), other *P. monacha-lucida* from the R0s Sinaloa (PA) and Fuerte (JA, SP, and CA), and *P. monacha* from the R0 Fuerte (JA).

Altogether, mitochondrial and allozyme data suggest the Mocerito hybridogen is closely related to R0 Fuerte *P. monacha-lucida* hemiclones, but the data do not pinpoint its geographical origin. Multiple hybrid origins have a reasonable probability of generating the same combined haplotype iden-

tified by limited number of allozyme and mitochondrial markers. This is especially true if alleles defining a hemiclone are relatively common in the sexual progenitors, which is the case for R0 Fuerte and R0 Sinaloa *P. monacha* (Vrijenhoek 1979b). Furthermore, R0 Sinaloa *P. monacha-lucida* should not be excluded from ancestry of the Mocerito hybridogen. Due to their remoteness, major regions of this river have not been sampled.

The discovery of a single male, *monacha* × *viriosa* hybrid among the *P. viriosa*-like fish examined in this study is fortunate because it confirmed a critical step (2) of the multistep scenario on which the reticulate origin hypothesis is built. The hybrid was heterozygous at each of six allozyme loci that distinguished *P. monacha* and *P. viriosa* (Table 2). Furthermore, its mtDNA haplotype was identical to that of the local *P. monacha-lucida*-like hybridogen (Fig. 3). The male possessed the genotype expected for an F₁ hybrid between a *P. monacha-lucida* female × *P. viriosa* male (e.g., MV in Fig. 1B).

DISCUSSION

Results of mitochondrial DNA analyses allowed us to reject the endemic and ancestral origin hypotheses for R0 Mocerito hybridogenetic females. The maternally inherited genome of these fish contained mtDNA sequences found in contemporary *P. monacha* and *P. monacha-lucida* from the R0s Fuerte and Sinaloa, neighboring river systems to the north. The data are completely consistent with the migrant origin hypothesis, namely that the Mocerito hybridogens are derived from *P. monacha-lucida* immigrants from the northern rivers, although the specific strain and place of origin could not be specified. Migration of *P. monacha-lucida* from northern rivers could have occurred via headwater stream capture or coastal stream capture. Distances between headwaters of the R0s Sinaloa and Fuerte and the R0s Sinaloa and Mocerito are very short at places, suggesting headwater capture as a possible mechanism for dispersal. Headwater capture has been reported in other species of *Poeciliopsis* (Mateos et al. 2002). Coastal stream capture is also a likely dispersal mechanism in this region of wide continental shelf, where opportunities for rivers to interconnect through estuaries or wide deltas are high, particularly during the Pleistocene low sea levels (Mateos et al. 2002).

Multilocus allozyme data also supported the migrant origin hypothesis, revealing no evidence for unique *viriosa* alleles in the hemiclone *M* genomes of the hybridogens. Nevertheless, we could not dismiss the possibility that *viriosa* alleles existed in the Mocerito hemiclone because *P. monacha* and *P. viriosa* share electromorphs at 23 of the 29 loci examined in this study. Allozymes only reveal similarity of net protein charges, not identity-by-descent. Without DNA sequences to assess the coancestry of these alleles, we could not discard the hypothesis that some of the shared electromorphs derived from *P. viriosa*.

Both allozyme and mtDNA data revealed that Mocerito hybridogens are not different from some R0 Fuerte *P. monacha-lucida* strains; however, the present result is not inconsistent with the multistep reticulate origin hypothesis posited by Vrijenhoek and Schultz (1974). Although no unique *vi-*

riosa allozymes were found in the hemiclone genome, only a limited number of diagnostic allozymes distinguish the closely related sibling-species, *P. monacha* and *P. viriosa* (Leslie 1982; Morizot et al. 1990). Furthermore, Vrijenhoek and Schultz (1974) argued, based on the results of crossing experiments, that the number of *viriosa* genes present in Mocerito hybridogens may be limited by strong genetic incompatibilities between *P. viriosa* and *P. lucida* genomes. Perhaps no *viriosa* genes exist in these hemiclone genomes. How then, can we explain the *viriosa*-like morphological traits (brassy coloration, black fin stripe, black tuxedo pattern, dorso-lateral scale counts, pre-orbital length) expressed by the Mocerito hemiclone examined by Vrijenhoek and Schultz? Can they be a consequence of phenotypic plasticity or evolutionary convergence? Environmentally induced phenotypic plasticity can be dismissed because the *viriosa* traits were clearly expressed in the laboratory-reared progeny from controlled crosses. Additionally, test crosses with *P. monacha* × *P. viriosa* hybrids demonstrated that these traits breed true (Vrijenhoek and Schultz 1974). Brassy coloration in *P. viriosa* is controlled by a single gene that affects the distribution of carotenoid-bearing pigment cells on the fins and body (Vrijenhoek 1976). Black fin stripe is controlled by macromelanophores concentrated between the first two dorsal fin rays. Black tuxedo pattern is sexually dimorphic and facultatively expressed during male courtship displays. The quantitative scalation and preorbital length traits that distinguish *P. monacha* and *P. viriosa* also are heritable. Evolutionary convergence for these traits cannot be excluded, but it seems unlikely that so many independent *viriosa*-like traits could arise by convergence in a *P. monacha-lucida* lineage that appears to be young based on the mitochondrial comparisons. Finally, it is possible that the wild-caught hemiclones we examined differed from the laboratory strain (S68-1 Cx, Univ. of Connecticut-Storrs) examined by Vrijenhoek and Schultz (1974). Perhaps the hemiclone represented by S68-1 Cx strain is now rare or extinct in the R0 Mocerito. The laboratory strain was terminated almost 10 years ago, and unfortunately, samples were not frozen or preserved for molecular analyses. Without repeating the quantitative genetic experiments of the earlier study with new Mocerito strains, we cannot be certain that current hybridogens carry the same *viriosa* characteristics. Nevertheless, the hybridogens examined in the present study expressed brassy body coloration, a *viriosa* trait that is not seen in *P. monacha-lucida* strains from the northern rivers.

Despite our uncertainty regarding strain identities, a fortuitous discovery provided support for the reticulate origin hypothesis. We identified an F₁ hybrid *monacha* × *viriosa* male from the R0 Mocerito with the same mtDNA haplotype (A) as the predominant Mocerito hemiclone. This finding supports prior observations that a few wild-caught Mocerito hybridogens produced *viriosa*-like broods of both sexes in the laboratory (Vrijenhoek and Schultz 1974). *Poeciliopsis* females can store sperm from multiple inseminations for several months (Leslie and Vrijenhoek 1977). Clearly, *P. monacha-lucida* females hybridize with local *P. viriosa* males and produce *monacha* × *viriosa* hybrids, a necessary step in reticulate origin hypothesis. Laboratory crosses revealed that Mendelian segregation and assortment and crossing over oc-

cur in F_1 *monacha* \times *viriosa* hybrids of both sexes (Leslie 1982; Morizot et al. 1990); therefore, generation of reticulate *m/v* genomes is likely in a natural hybrid swarm. Because a *viriosa* genome alone is incompatible with *lucida*, crosses of F_1 *monacha* \times *viriosa* hybrids with *P. lucida* males should favor recombinant *m/v* genomes with a limited number of *viriosa* genes. Feasibility of this step was demonstrated in the laboratory in 1971 with crosses that produced a novel hybridogenetic strain (Vrijenhoek and Schultz 1974). Unfortunately, the strain also was no longer available for genetic analyses.

The occurrence of *monacha* \times *viriosa* hybrids in the Río Mocorito suggests that hybridogens may serve as a vehicle for introgression of *monacha* genes into the local *P. viriosa* gene pool. The role of unisexual hybrids as conduits for interspecific gene flow is also suspected in unisexual salamanders and frogs (Uzzell et al. 1977; Uzzell 1982; Bogart 1989). Although our present mtDNA and allozymes data revealed no evidence for introgression of *P. monacha* genes into the *P. viriosa* gene pool, we need to examine populations of *P. viriosa* from rivers south of the Mocorito, where hybridogenetic fish do not exist.

Based on the molecular data alone, we have no evidence that R \circ Mocorito hybridogens differ substantially from contemporary R \circ Fuerte and R \circ Sinaloa *P. monacha-lucida* hemiclones. Thus, the Mocorito hybridogens also should be called *P. monacha-lucida*. Low mtDNA diversity found between the two Mocorito hemiclones could have arisen by mutation within a single lineage (i.e., postformational mutations). Alternatively, they might have arisen independently from genetically similar *P. monacha-lucida* immigrants. Our study provides one more example of relatively young asexual vertebrate lineages, further supporting the hypothesis that most asexual organisms are evolutionarily short-lived.

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LITERATURE CITED

- Avise, J. C., and R. C. Vrijenhoek. 1987. Mode of inheritance and variation of mitochondrial DNA in hybridogenetic fishes of the genus *Poeciliopsis*. *Mol. Biol. Evol.* 4:514–525.
- Avise, J. C., J. M. Quattro, and R. C. Vrijenhoek. 1992. Molecular clones within organismal clones. *Evol. Biol.* 26:225–246.
- Beukeboom, L., and R. C. Vrijenhoek. 1998. Evolutionary genetics and ecology of sperm-dependent parthenogenesis. *J. Evol. Biol.* 11:755–782.
- Bogart, J. P. 1989. A mechanism for interspecific gene exchange via all-female salamander hybrids. Pp. 170–179 in R. M. Dawley and J. P. Bogart, eds. *Evolution and ecology of unisexual vertebrates*. Bulletin no. 466. New York State Museum, Albany, NY.
- Bogart, J. P., and L. E. Licht. 1986. Reproduction and the origin of polyploids in hybrid salamanders of the genus *Ambystoma*. *Can. J. Genet. Cytol.* 28:605–617.
- Bulger, A. J., and R. J. Schultz. 1982. Origins of thermal adaptation in northern vs. southern populations of a unisexual hybrid fish. *Evolution* 36:1041–1050.
- Carmona, J. A., O. I. Sanjur, I. Doadrio, A. Machurdom, and R. C. Vrijenhoek. 1997. Hybridogenetic reproduction and maternal ancestry of polyploid Iberian fish: the *Tropidophoxinellus alburnoides* complex. *Genetics* 146:983–993.
- Cimino, M. C. 1972. Egg production, polyploidization and evolution in a diploid all-female fish of the genus *Poeciliopsis*. *Evolution* 26:294–306.
- Darlington, C. D. 1939. *The evolution of genetic systems*. Cambridge Univ. Press, Cambridge, U.K.
- Dawley, R. M., and K. A. Goddard. 1988. Diploid-triploid mosaics in unisexual hybrids of *Phoxinus eos* and *Phoxinus neogaeus*. *Evolution* 42:649–659.
- Hebert, P. D. N., and M. Beaton. 1993. *Methodologies for allozyme analysis using cellulose acetate electrophoresis*. Helena Laboratories, Beaumont, TX.
- Hedges, S. B., J. P. Bogart, and L. R. Maxson. 1992. Ancestry of unisexual salamanders. *Nature* 356:708–710.
- Judson, O. P., and B. B. Normark. 1996. Ancient asexual scandals. *Trends Ecol. Evol.* 11:41–46.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–120.
- Leslie, J. F. 1982. Linkage analysis of seventeen loci in the poeciliid fish genus *Poeciliopsis*. *J. Hered.* 73:19–23.
- Leslie, J. F., and R. C. Vrijenhoek. 1977. Genetic analysis of natural populations of *Poeciliopsis monacha*. *J. Hered.* 68:301–306.
- Mantovani, B., and V. Scali. 1992. Hybridogenesis and androgenesis in the stick-insect *Bacillus rossius-grandii benazzii* (Insecta, Phasmatodea). *Evolution* 46:783–796.
- Mark Welch, D., and M. Meselson. 2000. Evidence for the evolution of Bdelloid rotifers without sexual reproduction of genetic exchange. *Science* 288:1211–1215.
- Mateos, M., O. I. Sanjur, and R. C. Vrijenhoek. 2002. Historical biogeography of the fish genus *Poeciliopsis* (Cyprinodontiformes). *Evolution* 56:972–984.
- Maynard Smith, J. 1978. *The evolution of sex*. Cambridge Univ. Press, Cambridge, U.K.
- Miller, R. R. 1960. Four new species of viviparous fishes, genus *Poeciliopsis* from northwestern Mexico. *Occas. Pap. Mus. Zool. Mich.* 433:1–9.
- Morizot, D. C., R. J. Schultz, and R. S. Wells. 1990. Assignment of six enzyme loci to multipoint linkage groups in fishes of the genus *Poeciliopsis* (Poeciliidae): designation of linkage groups III–V. *Biochem. Genet.* 28:83–95.
- Murphy, R. W., J. Sites, D. G. Buth, and C. H. Haufler. 1996. *Proteins I: isozyme electrophoresis*. Pp. 51–120 in D. M. Hillis, C. Moritz, and B. K. Mable, eds. *Molecular systematics*. Sinauer, Sunderland, MA.
- Quattro, J. M., J. C. Avise, and R. C. Vrijenhoek. 1991. Molecular evidence for multiple origins of hybridogenetic fish clones (Poeciliidae: *Poeciliopsis*). *Genetics* 127:391–398.
- . 1992. An ancient clonal lineage in the fish genus *Poeciliopsis* (Atheriniformes: Poeciliidae). *Proc. Natl. Acad. Sci. USA* 89:348–352.
- Rasch, E. M., and J. S. Balsano. 1989. Trihybrids related to the unisexual molly fish, *Poecilia formosa*. Pp. 252–267 in R. M. Dawley and J. P. Bogart, eds. *Evolution and ecology of unisexual vertebrates*. Bulletin no. 466. New York State Museum, Albany, NY.
- Richardson, B. J., P. R. Baverstock, and M. Adams. 1986. *Allozyme electrophoresis: a handbook for animal systematics and population studies*. Academic Press, New York.
- Schartl, M., I. Nanda, I. Schlupp, B. Wilde, J. T. Epplen, M. Schmid, and J. Parzefall. 1995. Incorporation of subgenomic amounts of

- DNA as compensation for mutational load in a gynogenetic fish. *Nature* 373:68–71.
- Schultz, R. J. 1966. Hybridization experiments with an all-female fish of the genus *Poeciliopsis*. *Biol. Bull.* 130:415–429.
- . 1969. Hybridization, unisexuality and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Am. Nat.* 103:605–619.
- . 1973. Unisexual fish: laboratory synthesis of a “species.” *Science* 179:180–181.
- . 1977. Evolution and ecology of unisexual fishes. *Evol. Biol.* 10:277–331.
- Semlitsch, R. D., H. Hotz, and G.-D. Guex. 1997. Competition among tadpoles of coexisting hemiclones of hybridogenetic *Rana esculenta*: support for the frozen niche variation model. *Evolution* 51:1249–1261.
- Spolsky, C. M., C. A. Phillips, and T. Uzzell. 1992. Antiquity of clonal salamander lineages revealed by mitochondrial DNA. *Nature* 356:706–708.
- Stanley, S. M. 1975. Clades versus clones in evolution: why we have sex. *Science* 190:382–383.
- Swofford, D. L. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, MA.
- Uzzell, T. 1982. Introgression and stabilization in western Palearctic species of water frogs. Pp. 275–293 in D. Mossakowski and G. Roth, eds. *Environmental adaptation and evolution*. Gustav Fischer, Stuttgart, Germany.
- Uzzell, T. M., and L. Berger. 1975. Electrophoretic phenotypes of *Rana lessonae*, *Rana ridibunda*, and their hybridogenetic associate *Rana esculenta*. *Proc. Acad. Nat. Sci. Phila.* 127:13–24.
- Uzzell, T., R. Günther, and L. Berger. 1977. *Rana ridibunda* and *Rana esculenta*: a leaky hybridogenetic system (Amphibia: Salientia). *Proc. Acad. Nat. Sci. Phila.* 12:147–171.
- Vrijenhoek, R. C. 1976. An allele affecting display coloration in the fish *Poeciliopsis viriosa*. *J. Hered.* 67:324–325.
- . 1979a. Factors affecting clonal diversity and coexistence. *Am. Zool.* 19:787–797.
- . 1979b. Genetics of a sexually reproducing fish in a highly fluctuating environment. *Am. Nat.* 113:17–29.
- . 1984a. Ecological differentiation among clones: the frozen niche variation model. Pp. 217–231 in K. Wöhrmann and V. Loeschcke, eds. *Population biology and evolution*. Springer, Heidelberg, Germany.
- . 1984b. The evolution of clonal diversity in *Poeciliopsis*. Pp. 399–429 in B. J. Turner, ed. *Evolutionary genetics of fishes*. Plenum Press, New York.
- . 1989. Genetic and ecological constraints on the origins and establishment of unisexual vertebrates. Pp. 24–31 in R. Dawley and J. Bogart, eds. *Evolution and ecology of unisexual vertebrates*. Bulletin no. 466. New York State Museum, Albany, NY.
- . 1993. The origin and evolution of clones versus the maintenance of sex in *Poeciliopsis*. *J. Hered.* 84:388–395.
- . 1998. Clonal organisms and the benefits of sex. Pp. 151–172 in G. Carvalho, ed. *Advances in molecular ecology*. IOS Press, Amsterdam.
- Vrijenhoek, R. C., and R. J. Schultz. 1974. Evolution of a trihybrid unisexual fish (*Poeciliopsis*, Poeciliidae). *Evolution* 28:205–319.
- Vrijenhoek, R. C., R. A. Angus, and R. J. Schultz. 1977. Variation and heterozygosity in sexually vs. clonally reproducing populations of *Poeciliopsis*. *Evolution* 31:767–781.
- . 1978. Variation and clonal structure in a unisexual fish. *Am. Natur.* 112:41–55.
- Williams, G. C. 1975. *Sex and evolution*. Princeton Univ. Press, Princeton, NJ.

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