Molecular Evidence for Multiple Origins of Hybridogenetic Fish Clones (Poeciliidae: *Poeciliopsis*)

Joseph M. Quattro,* John C. Avise† and Robert C. Vrijenhoek*

**Center for Theoretical and Applied Genetics, Cook College, Rutgers University, New Brunswick, New Jersey 08903–0231, and †Department of Genetics, University of Georgia, Athens, Georgia 30602*

Manuscript received September 6, 1990
Accepted for publication October 11, 1990

ABSTRACT

Hybrid matings between the sexual species *Poeciliopsis monacha* and *Poeciliopsis lucida* produced a series of diploid all-female lineages of *P. monacha-lucida* that inhabit the Río Fuerte of northwestern Mexico. Restriction site analyses of mitochondrial DNA (mtDNA) clearly revealed that *P. monacha* was the maternal ancestor of these hybrids. The high level of mtDNA diversity in *P. monacha* was mirrored by similarly high levels in *P. monacha-lucida*; thus hybridizations giving rise to unisexual lineages have occurred many times. However, mtDNA variability among *P. monacha-lucida* lineages revealed a geographical component. Apparently the opportunity for the establishment of unisexual lineages varies among tributaries of the Río Fuerte. We hypothesize that a dynamic complex of sexual and clonal fishes appear to participate in a feedback process that maintains genetic diversity in both the sexual and asexual components.

GENETIC studies have revealed that clonally reproducing, all-female "species" of vertebrates are the products of hybridization between congeneric sexual species (SCHULTZ 1969; SITES *et al.* 1990; VRIJENHOEK *et al.* 1989). A new unisexual (all-female) lineage can be established if genetic differences between the hybridizing entities are sufficient to disrupt recombinant processes during meiosis, without severely limiting viability, fecundity, and other characteristics affecting fitness of the hybrids (MORITZ *et al.* 1989; VRIJENHOEK 1989). With such a narrow window of opportunity, it is not surprising that the origins and establishment of new unisexual vertebrates have been ecologically, geographically, and phylogenetically constrained (DENSMORE *et al.* 1989; DENSMORE, WRIGHT and BROWN 1989; MORITZ, WRIGHT and BROWN 1989; VRIJENHOEK 1989; VYAS *et al.* 1990).

An exception might be unisexual fishes of the genus *Poeciliopsis* which have arisen independently in several river systems of Sonora and Sinaloa, Mexico. Hybridization between *P. monacha* and several sexual species in this genus has produced allodiploid and allotriploid unisexual biotypes (SCHULTZ 1977). In this study, we focus on the allodiploid biotype *P. monacha-lucida* (designated ML), which reproduces by hybridogenesis, a hemiclonal mechanism that transmits only the *monacha* (M) chromosome set to developing ova (SCHULTZ 1969). The *lucida* (L) chromosomes are excluded during a premeiotic cell division, precluding synapsis and crossing over (CIMINO 1972). Sperm from *P. lucida* males is required for fertilization and restoration of diploidy.

Protein electrophoresis uncovered considerable diversity among the hemiclonal *monacha* genomes of *P. monacha-lucida* from the Río Fuerte (VRIJENHOEK, ANGUS and SCHULTZ 1977). For brevity, we refer to distinct hemiclones defined by electrophoresis as "E-types" and identify them by the Roman numerals following the biotype designation (*e.g.*, ML/VIII). All the allozymic diversity distinguishing Río Fuerte hemiclones also exists in *P. monacha* from this river. However, endemic allozymes, found only in discrete *P. monacha* populations, often mark co-occurring hemiclones, suggesting that these ML lineages arose during independent hybridization events in partially isolated tributaries.

Subsequent tissue grafting studies of Río Fuerte *P. monacha-lucida* identified 18 histocompatibility clones (H-types) hidden within the eight E-types (ANGUS and SCHULTZ 1979). What proportion of this additional variation was due to multiple hybridization events *vs.* postformational mutations within E-type lineages could not be determined. However, evidence for postformational divergence was found in expanded electrophoretic surveys that included more loci and unisexual fish from additional river systems (SPINELLA and VRIJENHOEK 1982; VRIJENHOEK 1984). Furthermore, genetic studies of several ML strains revealed that hemiclonal M genomes carried recessive lethals and subvitals that probably arose post-formationally (LESLIE and VRIJENHOEK 1978, 1980). Apparently, some hybridogenetic strains have existed long enough to accumulate a substantial load of mutations, but the data could not be used to infer relative evolutionary ages of hemiclonal lineages.

Restriction site analysis of mitochondrial DNA has

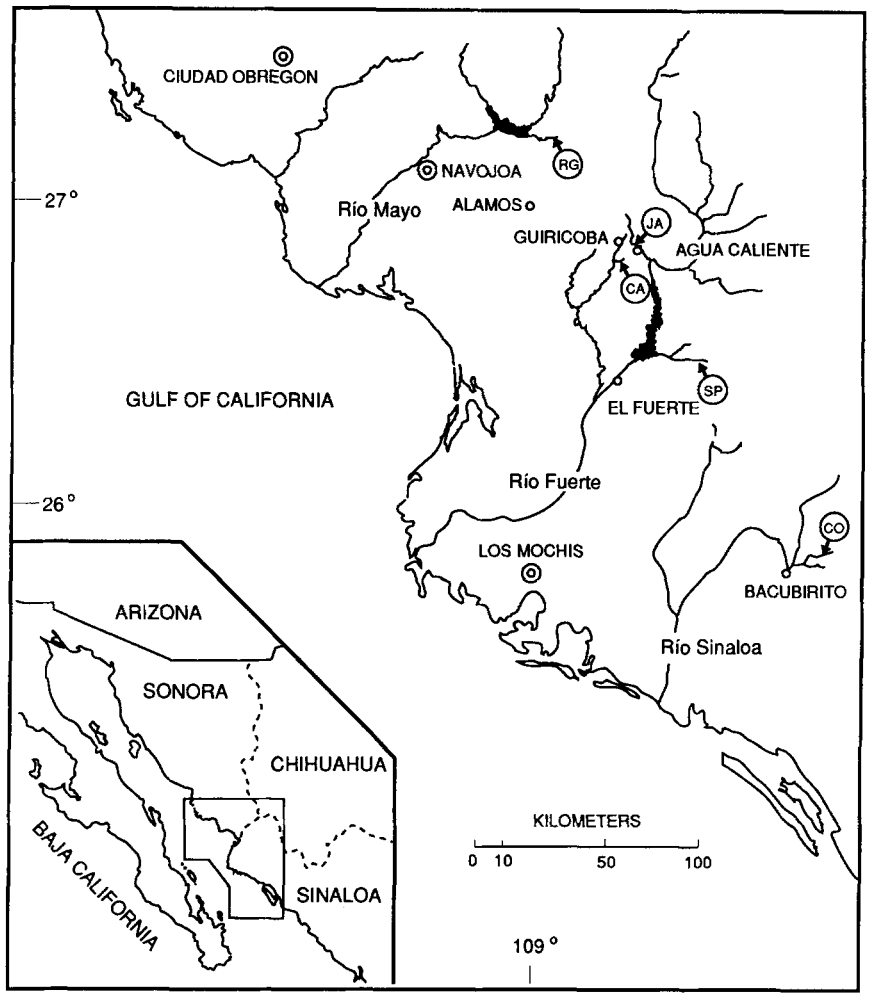

FIGURE 1.—The Ríos Fuerte, Mayo, and Sinaloa of northern Mexico and associated tributaries. Sampling localities (arrows) are indicated by site abbreviations: CA, the Arroyo Aguajita de El Cajon; JA, the Arroyo de Jaguari and its feeder streams (Arroyos de los Platanos and Nachapulon); SP, Rio San Pedro; RG, Rancho Guamúchil; and CO, Arroyo Seco *ca.* Coronado. Refer to text for more detailed site descriptions.

become a useful tool for studying the origins and evolutionary ages of unisexual vertebrates (MORITZ, DOWLING and BROWN 1987). Because it is maternally inherited, mtDNA contains in its sequence the evolutionary history of an all-female lineage. Additionally, the molecule's rapid rate of sequence evolution facilitates microevolutionary studies below the species level. A preliminary analysis of mtDNA restriction fragment polymorphism in Poeciliopsis (AVISE and VRIJENHOEK 1987), revealed three points relevant to the present study. First, *P. monacha* and *P. lucida* have widely divergent mtDNAs, allowing unequivocal identification of *P. monacha* as the maternal progenitor of two naturally occurring *P. monacha-lucida* strains (ML/VII and ML/VIII). Second, these natural hybridogens, plus five laboratory strains, clearly demonstrated that mtDNA is maternally inherited. Finally, this study revealed no evidence that the natural strains had diverged mutationally from one another or from the coexisting *P. monacha* population.

In the present study, we conducted a survey of mtDNA diversity in *P. monacha*, the common ancestor of all unisexual forms of Poeciliopsis, and compared its variation to that found in *P. monacha-lucida* hybridogens from the Río Fuerte. By integrating information from allozymes, tissue grafting, and mtDNA we address the following questions: (1) is *P. monacha* the maternal progenitor to all of *P. monacha-lucida* lineages; (2) are certain *P. monacha* populations more prone to producing unisexual lineages; and (3) is there evidence for mutationally divergent, potentially long-lived unisexual lineages. Finally, we comment on the recent decline of *P. monacha* populations in three river systems encompassing its complete range, and hopefully raise concern regarding conservation of the common ancestor to all unisexual lineages in the genus *Poeciliopsis*.

MATERIALS AND METHODS

Poeciliopsis monacha was originally described as having a relictual distribution in headwater tributaries of the Ríos Fuerte, Mayo, and Sinaloa of northwestern Mexico (MILLER 1960; VRIJENHOEK 1979b). Prior to 1978, *P. monacha* were found at a single locality in the Río Sinaloa (Arroyo Seco, ca. Rancho Coronado, Sinaloa). It might be extinct in this river, since all subsequent attempts to capture this species were unsuccessful. No laboratory strains from this population were available for study. Prior to 1989, *P. monacha* were found at two sites in the Guamúchil tributary of Río Mayo (*ca.* El Tabelo and *ca.* Rancho Guamúchil, Sonora).

TABLE 1

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

Locality and biotype	Strain: Wild	Strain: Laboratory	E-type[a]	Mt-type[b]
Rio Fuerte				
A. San Pedro				
P. monacha	1			2
P. monacha	1			8
P. monacha	1			11
P. monacha	1			14
P. monacha-lucida	2		I	11
P. monacha-lucida	1		II	14
P. monacha-lucida	1		IV	7
B. Guiricoba				
P. monacha	6			7
P. monacha-lucida	1		I	11
P. monacha-lucida	1		I	12
P. monacha-lucida	1		II	13
P. monacha-lucida	4		II	14
P. monacha-lucida	1		III	9
P. monacha-lucida		1	IV	9
P. monacha-lucida	1		XV	10
C. Jaguari				
P. monacha	3	3		1
P. monacha	1			3
P. monacha	4			7
P. monacha-lucida	2	1	VII	1
P. monacha-lucida	2	1	VIII	1
Rio Mayo				
P. monacha		1		5
P. monacha		2		6

[a] Refer to Table 3.
[b] Refer to Table 2.

Recent attempts to capture this species at these sites have been unsuccessful. Fortunately three independent isofemale lineages from Rancho Guamúchil were available for the present analysis. Headwater tributaries of the Río Fuerte still maintain *P monacha* populations. We examined 18 wild-caught females and three isofemale lines from three headwater tributaries in this river (Figure 1 and Table 1): the Arroyo Aguajita de El Cajón which connects with the Arroyo de Guiricoba (*ca.* El Cajón, Sonora); the Arroyo de Jaguari and its feeder streams (Arroyos de los Platanos and Nachapulon; *ca.* Agua Caliente, Sonora); and the Rio San Pedro (approximately 22 km upstream from the San Pedro, Sonora). mtDNA patterns in the sexual species were compared with those of three isofemale strains and 17 wild females of *P. monacha-lucida* from the same localities.

Morphological phenotypes were used to distinguish *P. monacha* from unisexual biotypes in the field (SCHULTZ 1969). Wild-caught specimens were returned to the culture facility at Rutgers University, where protein electrophoresis was used to distinguish among diploid and triploid biotypes. Electrophoretic techniques, diagnostic genotypes, and gene dosage patterns for 25 loci were described in earlier publications (VRIJENHOEK 1975; VRIJENHOEK, ANGUS and SCHULTZ 1977, 1978). For the present study, we added a 26th locus, *Pep-lgg* (leucyl-glycyl-glycine peptidase EC 3.4.11) (for methods see MULVEY and VRIJENHOEK 1981).

Mitochondrial DNA was isolated from fresh tissues following the methods of LANSMAN *et al.* (1983). Because of low yields of mtDNA, we used only fresh female specimens ≥25 mm standard length. Purified mtDNAs were digested according to the manufacturers' specifications with a battery of 17 restriction endonucleases having four (*Msp*I), five (*Ava*I, *Ava*II, *Hin*cII), and six (*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) base recognition sequences. DNA fragments were end-labeled with [^{35}S]dNTP(s) and separated in 0.8–1.8% agarose gels. After electrophoresis, gels were dried under vacuum onto a filter paper backing and exposed to X-ray film at room temperature for 24–72 hr.

Each restriction digest profile for a specific endonuclease was given an arbitrary letter code. Composite scores, representing a letter code for each of 17 gel-fragment profiles, were assigned to each individual. Since all gel-fragment patterns could be related by specific site gains or losses, a matrix of the minimum number of site changes necessary to interrelate composite haplotypes was constructed. A minimum length network linking observed haplotypes was constructed by inputting the mutation matrix into the minimum spanning tree algorithm of the NTSYS statistical package (ROHLF, KISHPAUGH and KIRK 1974). The resulting network was compared to the consensus of the most parsimonious trees obtained with the CONSENSE and MIX computer programs (FELSENSTEIN 1990). We evaluated the fit of the resulting network to the input matrix by the Mantel matrix comparison test (SMOUSE, LONG and SOKAL 1986). The significance of the resulting matrix correlation coefficient was evaluated by comparison to calculated test statistics after 1000 random permutations of the input matrix. Nucleon diversity, h, a measure of mtDNA haplotype variability, was calculated according to the formula of NEI and TAJIMA (1981).

RESULTS

Ten restriction endonucleases produced polymorphic fragment profiles, revealing 13 mtDNA haplotypes among the 44 specimens. Divergence among these 13 haplotypes could be attributed to changes at 16 restriction sites (Table 2). The minimum length network interrelating these haplotypes is illustrated in Figure 2. The total number of required mutational steps along branches of the network connecting haplotypes (320) is slightly larger than the minimum number inferred from the composite haplotypes (316), indicating only slight distortion in the network (normalized Mantel Z statistic = 0.99, p{random $Z \geq$ observed Z} = 0.001). The distortion is attributable to two instances of homoplasy: parallel losses of the *Ava*I[a] site in haplotypes 12 and 14, and parallel gains of the *Bst*EII[g] site in haplotypes 1 and 6 (Table 2, Figure 2).

Considerable mtDNA diversity was found in *P. monacha* (Table 1). Seven haplotypes were observed among 18 wild females and three isofemale lines from the Río Fuerte (nucleon diversity: $h = 0.72 \pm 0.06$), and two unique haplotypes were observed among the three Río Mayo lines. The eight Río Fuerte haplotypes were not evenly distributed among the three localities. The most common variant (haplotype 7) was fixed (100%) in the Guiricoba sample of *P. monacha*; it occurred at lower frequency in the Jaguari sample (36%), and was absent from the San Pedro sample.

TABLE 2

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

	Restriction site															
	*Ava*I	*Ava*II				*Bst*EII		*Eco*RI	*Hinc*II	*Hin*dIII			*Msp*I	*Pst*I	*Xba*I	*Spe*I
Haplotype	a	b	c	d	e	f	g	h	i	j	k	l	n	o	p	q
1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0
2	1	1	0	0	0	0	0	1	0	1	1	1	1	0	0	0
3	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0
5	1	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0
6	1	1	0	0	0	0	1	0	1	1	1	1	0	1	0	0
7	1	1	0	0	0	0	0	1	0	1	1	1	1	0	0	1
8	1	1	0	0	0	0	0	1	0	0	1	1	1	0	0	1
9	1	1	1	0	0	0	0	1	0	1	1	0	1	0	1	0
10	1	0	0	0	0	0	0	1	0	1	1	1	1	0	0	1
11	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	1
12	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	1
13	1	1	0	1	1	0	0	1	0	1	1	1	1	0	0	0
14	0	1	0	1	1	0	0	1	0	1	1	1	1	0	0	0

A minimum length network based on these data is shown in Figure 2.

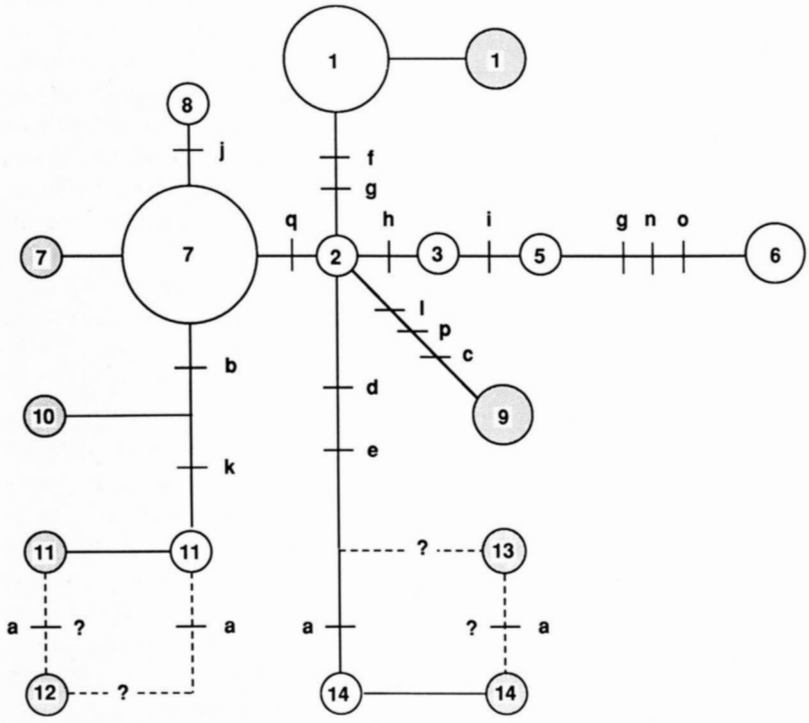

FIGURE 2.—An unrooted minimum length network summarizing relationships among observed mtDNA haplotypes in sexual *Poeciliopsis monacha* (open circles) and unisexual *P. monacha-lucida* (stippled circles). For *P. monacha*, circles are drawn in proportion to the observed number of individuals with the respective haplotype. *P. monacha-lucida* circles are proportional to the number of different electromorph clones observed with the respective haplotype. Slashes represent the number of mutations necessary to interrelate haplotypes, and letter codes refer to specific restriction sites in Table 2. The boxes and dashed lines are alternative pathways in the network, and are explained in the text. Haplotype 4, which occurs only in triploid unisexual Poeciliopsis, is not shown, and will be described elsewhere (J. M. QUATTRO, unpublished results).

Haplotype 1 characterized 55% of the Jaguari sample, but was not found elsewhere. Remarkably, four unique haplotypes were found in a sample of four *P. monacha* females from the Río San Pedro (haplotypes 2, 8, 11 and 14).

Sequence divergence between *P. monacha* and *P. lucida* mtDNA is extensive (p = 11.5%) (AVISE and VRIJENHOEK 1987). Fragment profiles for all restriction enzymes examined in this and the previous study diagnostically differentiate between the two species. Thus we could unequivocally assign maternal parentage to the unisexual samples examined in this study. All *P. monacha-lucida* females contained *monacha* type mtDNAs that were identical with or most closely related to Río Fuerte *P. monacha* haplotypes (Figure 2). Río Mayo *P. monacha* haplotypes are distinguished by a unique *Hinc*II^{i} fragment profile.

Addition of the *Pep-lgg* locus to the earlier suite of 25 gene loci (VRIJENHOEK 1984; VRIJENHOEK, ANGUS and SCHULTZ 1978) identified a new E-type (ML/XV) from the Arroyo de Guiricoba. Composite allozyme genotypes of the seven E-types observed in this study are listed in Table 3. All the allozymes discriminating among E-types are encoded by common alleles in *P. monacha* (*cf.* VRIJENHOEK 1979b). The seven E-types exhibited eight distinct mtDNA haplotypes, or Mt-types (Table 1). Some E-types could be decomposed into distinct Mt-types, for example: ML/I was marked

TABLE 3

Allozyme haplotypes (E-types) of the *monacha* genome in *P. monacha-lucida*

E-type	Diagnostic loci					
	Aat-3	*Est-5*	*Ldh-1*	*Ck-A*[a]	*Pep-lgg*	*Pgd*
I	*b*	*f*	*b*	*a*	*a*	*a*
II	*b*	*c*	*b*	*a*	*a*	*a*
III	*a*	*c*	*b*	*a*	*a*	*a*
IV	*b*	*d*	*b*	*a*	*b*	*a*
VII	*b*	*e*	*a*	*b*	*a*	*a*
VIII	*b*	*d*	*a*	*a*	*a*	*c*
XV	*b*	*f*	*b*	*a*	*b*	*a*

[a] Previously called *Mp-3* because it encodes a soluble muscle protein (VRIJENHOEK, ANGUS and SCHULTZ 1978).

by Mt-types 11 and 12; ML/II by Mt-types 13 and 14; and ML/IV by Mt-types 7 and 9. Conversely, some E-types carried identical mtDNA haplotypes (*e.g.*, ML/VII and ML/VIII carried Mt-type 1; ML/III and ML/IV carried Mt-type 9). To facilitate identification of these hemiclones, we append the Mt-type to the E-type designations (*e.g.*, ML/IV.7 *vs.* ML/IV.9).

The numbers of sexual and hybridogenetic fish in the sample were similar (24 *vs.* 20, respectively). Four of the mtDNA variants found in *P. monacha-lucida* also were observed in the sample of *P. monacha* (haplotypes 1, 7, 11 and 14), and four others (9, 10, 12 and 13) were not. Mt-type 9 differed by three unique mutations from the nearest *P. monacha* haplotype (2), but it was found in two distinct E-types sampled from the Guiricoba locality, ML/III.9 and ML/IV.9. The E-types of these hemiclones differ with respect to common *P. monacha* allozymes at three polymorphic loci (Table 3), presumably as a result of independent origins. Thus, mtDNA haplotype 9 must have existed in *P. monacha* prior to the origin of these hemiclonal lineages. Mt-types 10, 12, and 13 of ML/XV.10, ML/I.12 and ML/II.13) each differed by a single mutation from the nearest *P. monacha* haplotypes in the network (Figure 2). These orphan haplotypes probably exist, or existed, in *P. monacha*; however, we cannot rule out the possibility that ML/I.12 and ML/II.13 represent one-step mutations within clonal lineages (represented as rectangles in Figure 2).

DISCUSSION

Given the large amount of sequence divergence between *P. monacha* and *P. lucida* mtDNAs (AVISE and VRIJENHOEK 1987), we could unequivocally assign maternal parentage to each unisexual specimen. *P. monacha* is the maternal progenitor of all the *P. monacha-lucida* hybridogens examined in this study. This finding confirms earlier laboratory studies, wherein matings of *P. monacha* females × *P. lucida* males sometimes produce viable hybridogenetic lineages (SCHULTZ 1973b), but the reciprocal matings do not (R. J. SCHULTZ, personal communication). As found previously with a small sample of hybridogenetic lineages (AVISE and VRIJENHOEK 1987), paternal leakage does not appear to occur in *P. monacha-lucida*. We found no evidence for heteroplasmy of *monacha* and *lucida* mtDNAs in any of the unisexual specimens.

Compared with other unisexual vertebrates, *P. monacha-lucida* is exceptional, because its mtDNA diversity essentially matches that of its maternal ancestor, *P. monacha*. In contrast, parthenogenetic lizards of the genera *Cnemidophorus* and *Heteronotia* are far less diverse than their closest sexual relatives (DENSMORE *et al.* 1989; DENSMORE, WRIGHT and BROWN 1989; MORITZ, WRIGHT and BROWN 1989). In general, parthenogenetic lizards appear to have arisen as a result of a limited number of hybridization events. Similarly, the gynogenetic fish *Menidia clarkhubbsi* exhibits low mtDNA diversity relative to its sexual relative *P. peninsulae* (ECHELLE *et al.* 1989), but multiple events were involved in producing different clonal lineages. MtDNA haplotypes in a limited sample of the gynogenetic fish *Phoxinus eos-neogaeus* also indicate multiple hybrid origins (GODDARD, DAWLEY and DOWLING 1989). Whether multiple origins are characteristic of the hybridogenetic waterfrog, *Rana esculenta*, is unclear, because the patterns of mating and mode of hemiclonal reproduction are more complex than in *Poeciliopsis* (SPOLSKY and UZZELL 1986).

The present data clearly corroborate earlier allozyme studies implicating multiple hybridization events as the primary source of hemiclonal diversity in *P. monacha-lucida* (VRIJENHOEK 1984; VRIJENHOEK, ANGUS and SCHULTZ 1978). However, use of mtDNA restriction site analyses alone might underestimate the opportunity for multiple origins in unisexual vertebrates. For example, a single hybridization of *P. monacha* × *P. lucida* would give rise to sibling *P. monacha-lucida* strains that differ in nuclear genotypes, but share identical mtDNAs (AVISE and VRIJENHOEK 1987; WETHERINGTON *et al.* 1989). Similarly, independent hybridizations involving separate *P. monacha* females from the same maternal lineage also give rise to such pairs.

Despite these conservative features of mtDNA, we were able to discriminate between clones marked by identical allozyme genotypes. For example, ML/IV.7 from the Río San Pedro and ML/IV.9 from the Guiricoba tributary carry distinct mtDNA haplotypes. It was previously shown that ML/IV E-types from these tributaries had distinct H-types (ANGUS and SCHULTZ 1979), but we could not ascertain whether the H-types arose independently. In this case, independent hybrid events are likely to capture identical E-types, because ML/IV contains the most frequent *P. monacha* alleles at all polymorphic loci (compare Table 3 with VRIJENHOEK 1979b). Mutational divergence of the mtDNAs

in ML/IV.7 and ML/IV.9 is less likely because these haplotypes are separated by at least four mutations. Unfortunately, strains used in the earlier tissue grafting studies were not maintained, preventing a comprehensive evaluation of relationships among E-types, H-types, and Mt-types.

Tissue grafting studies revealed that some *P. monacha-lucida* hemiclones are widely distributed in the Río Fuerte (ANGUS and SCHULTZ 1979). We found hemiclone ML/I.11 in both the San Pedro and Guiricoba tributaries, more than 150 km apart. Similarly, hemiclone ML/II.14 was captured at both sites. Given the high diversity of allozyme and mtDNA genotypes in *P. monacha*, it is unlikely that two hemiclones would freeze identical nuclear and mitochondrial genotypes during independent origins. Furthermore, mtDNA haplotypes 11 and 14 were not observed in *P. monacha* from Guiricoba locality. Although we cannot exclude the possibility that these haplotypes were once more broadly distributed in *P. monacha*, it is more parsimonious to propose that ML/I.11 and ML/II.14 each had single origins and then spread to other sites in the Río Fuerte.

Conversely, other hemiclones appear endemic to certain tributaries of the Río Fuerte. For example, hemiclones ML/VII.1 and ML/VIII.1 bear allozymes and mtDNA haplotypes that are unique to *P. monacha* from the Jaguari tributary and its feeder streams. With diallelic polymorphisms at five loci in *P. monacha* from this tributary, we have the capacity to identify 32 E-types at this site (VRIJENHOEK, ANGUS and SCHULTZ 1978), but only ML/VII and ML/VIII have been found in surveys involving several thousand individuals over a 15-yr period (SCHENCK and VRIJENHOEK 1986). The present addition of three mtDNA haplotypes to the suite of characters found in *P. monacha* at this site raises the number of possible hemiclonal combinations to 96, but still we found only two. Similarly, tissue grafting studies identified no additional H-type variation beyond the major differences defining ML/VII and ML/VIII (ANGUS and SCHULTZ 1979). Hybridization experiments involving *P. monacha* females from the Jaguari tributary were less likely to produce viable hybridogenetic lineages than females from the San Pedro or Guiricoba tributaries (WETHERINGTON, KOTORA and VRIJENHOEK 1987). Perhaps opportunities for the origin and establishment of new hybrid lineages are limited at this site.

Hybrid origins appear to be more likely in the Río San Pedro than in other tributaries of the Río Fuerte. Comparatively more mtDNA haplotypes found in *P. monacha-lucida* were associated with *P. monacha* haplotypes sampled from the San Pedro than either the Guiricoba or Jaguari populations. This conclusion is supported by the laboratory hybridization experiments (WETHERINGTON, KOTORA and VRIJENHOEK 1987). *P. monacha* females from the Río San Pedro were more likely to yield viable and fertile hybridogenetic lineages than *P. monacha* from any other region within the Río Fuerte.

Surveys of allozyme diversity in hybridogens from other rivers also revealed endemic hemiclones marked by local *P. monacha* allozymes. The Río Sinaloa, just south of the Río Fuerte, has several endemic hemiclones of *P. monacha-lucida* (VRIJENHOEK 1984). Similarly, the Río Mayo, just north of the Río Fuerte, has endemic hemiclones of *P. monacha-occidentalis* (VRIJENHOEK, ANGUS and SCHULTZ 1977). Clearly, multiple independent origins of hybridogenetic strains have occurred within all major drainage systems that contain *P. monacha*.

Although multiple independent origins explain most of the mtDNA diversity in *P. monacha-lucida*, mutations might also have contributed. Some *P. monacha-lucida* individuals have identical allozyme genotypes but harbor mtDNA variants differing by a single mutation. We previously argued that independent origins could produce hemiclones with identical allozyme genotypes but distinct mtDNAs, yet in some cases post-formational mutation of mtDNA within a hemiclonal lineage also seems likely. For example, the mtDNAs of E-type ML/I.11 and ML/I.12 differ by a single mutation. ML/I.12 could have arisen directly from ML/I.11, or it might have arisen independently from a *P. monacha* lineage harboring mtDNA haplotype 12 that was not included in our sample (Figure 2). Similarly possibilities exist for the hemiclonal pair ML.II.13 and ML/II.14. Both the ML/I and ML/II E-types are composed of common alleles segregating in Rio Fuerte *P. monacha*; thus, independent origins, giving rise to identical E-types are likely.

With the limited sample available for this study, and the shrinking number of *P. monacha* populations remaining in nature, we cannot easily determine how important mutation within hemiclones has been as a source of mtDNA diversity for these fish. Despite the indirect evidence for postformational mutation in hemiclonal mtDNAs and in enzyme-determining gene loci (SPINELLA and VRIJENHOEK 1982; VRIJENHOEK 1984), genetic differences do not go beyond the broad variation segregating in extant populations of *P. monacha*. Thus, the origins of hemiclonal Poeciliopsis must have occurred within the time scale of matriarchal lineage diversification in *P. monacha*. Without exception, molecular genetic studies of unisexual vertebrates have concluded that the unisexual populations are recently evolved (DENSMORE *et al.* 1989; DENSMORE, WRIGHT and BROWN 1989; ECHELLE *et al.* 1989; GODDARD, DAWLEY and DOWLING 1989; MORITZ *et al.* 1989; MORITZ, WRIGHT and BROWN 1989).

We find it curious that *P. monacha*, a sexual species characterized by relatively small and highly subdi-

vided relictual populations, contains so much genetic diversity. It was previously hypothesized that the sexual species might recapture variation through rare hybridizations with the widely dispersed and more abundant hybridogens (VRIJENHOEK 1979b). Hemiclonal variation within *P. monacha-lucida* populations can enter the sexual gene pool if hybridogens mate with *P. monacha* males (*e.g.*, M′L × MM). The resulting M′M progeny, if viable and fertile, reproduce sexually and have normal meiosis (LESLIE and VRIJENHOEK 1980; SCHULTZ 1973a; VRIJENHOEK and SCHULTZ 1974). Thus, *P. monacha-lucida* might serve as a reservoir of genetic variation, connecting isolated populations of *P. monacha*, and buffering the loss of nuclear and organeller variation.

Circumstantially, we found mtDNA haplotypes in *P. monacha-lucida* that might be intermediates between sexual haplotypes. For example, ML/XV.10 might connect haplotypes 7 and 11 in *P. monacha* (Figure 2). Alternatively, haplotype 10 might have been frozen from a *P. monacha* lineage that we failed to sample, or has since disappeared. Although we cannot demonstrate the feedback process with the present data, its feasibility has been shown clearly in the laboratory (LESLIE and VRIJENHOEK 1980), and we suspect it also occurs in nature. Ultimately, we must view *monacha* genomes as dynamic assemblages of genes that exist in at least two states: as recombining units in a sexual species and as potentially recombining units frozen in the hemiclonal genomes of the hybridogens.

It is unfortunate that *P. monacha*, the common sexual ancestor to all the known unisexual-hybrid biotypes of *Poeciliopsis*, is presently in danger of extinction. It might already be extinct in the Ríos Mayo and Sinaloa, and it is seriously threatened in the San Pedro and Guiricoba tributaries, where native habitats have been altered with the introduction of exotic fishes, primarily *Tilapia* spp. If *P. monacha* disappears from a river, the opportunity for *de novo* origins of hybridogenetic lineages also disappears. Previous studies revealed that the ecological health of unisexual populations depends on the opportunity for *de novo* hybrid origins and interclonal selection (VRIJENHOEK 1979a; WETHERINGTON, KOTORA and VRIJENHOEK 1987; WETHERINGTON, SCHENCK and VRIJENHOEK 1989; WETHERINGTON *et al.* 1989). Ironically, just as variability in hemiclonal populations depends on variation frozen from *P. monacha*, variability in *P. monacha* might also depend on feedback from the genetic reservoir in the unisexual populations. We should seek means to preserve all components of this dynamic genetic complex.

We gratefully acknowledge the staff of the Centro Ecologico de Sonora, Hermosillo, Mexico, for their collaboration and hospitality, particularly LOUDES JUÁREZ-ROMERO, ALEJANDRO VARELA ROMERO and JOSÉ CAMPOY-FAVELA. Also, we thank DEAN A. HENDRICKSON for help in the field and BILL NELSON for assistance in the laboratory. Specimens used in this study were collected under permits #412.2.1.3.0 folio 4815 issued jointly by the Departmento de Pesca, by Secretaria de Desarrollo Urbano y Ecologia, and by the Departamento de Exteriores, Mexico, D.F. ANGELICA NARVAEZ, Office of the U.S. Science Attache, Mexico, was instrumental in obtaining these permits. Research was supported by grants BSR88-05360 (J.C.A.) and BSR88-05361 (R.C.V.) from the National Science Foundation, the Theodore Roosevelt Memorial Fund, American Museum of Natural History (J.M.Q.), and by the Leatham-Steinetz-Stauber Graduate Research Fund, Rutgers University (J.M.Q).

LITERATURE CITED

ANGUS, R. A., and R. J. SCHULTZ, 1979 Clonal diversity in the unisexual fish *Poeciliopsis monacha-lucida*: a tissue graft analysis. Evolution **33:** 27–40.

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.

CIMINO, M. C., 1972 Egg production, polyploidization and evolution in a diploid all-female fish of the genus *Poeciliopsis*. Evolution **26:** 294–306.

DENSMORE, L. D., III., J. W. WRIGHT and W. M. BROWN, 1989 Mitochondrial-DNA analyses and the origin and relative age of parthenogenetic lizards (genus *Cnemidophorus*). II. *C. neomexicanus* and the *C. tesselatus* complex. Evolution **43:** 943–957.

DENSMORE, L. D., III., C. C. MORITZ, J. W. WRIGHT and W. M. BROWN, 1989 Mitochondrial-DNA analyses and the origin and relative age of parthenogenetic lizards (genus *Cnemidophorus*). IV. Nine *sexlineatus*-group unisexuals. Evolution **43:** 969–983.

ECHELLE, A. A., T. E. DOWLING, C. C. MORITZ and W. M. BROWN, 1989 Mitochondrial-DNA diversity and the origin of the *Menidia clarkhubbsi* complex of unisexual fishes (Atherinidae). Evolution **43:** 984–993.

FELSENSTEIN, J., 1990 *PHYLIP Manual Version 3.3.* University Herbarium, University of California, Berkeley, Calif.

GODDARD, K. A., R. M. DAWLEY and T. E. DOWLING, 1989 Origin and genetic relationships of diploid, triploid, and diploid-triploid mosaic biotypes in the *Phoxinus eos-neogaeus* unisexual complex, pp. 268–280 in *Evolution and Ecology of Unisexual Vertebrates*, edited by R. DAWLEY and J. BOGART. New York State Museum, Albany, N.Y.

LANSMAN, R. A., R. O. SHADE, J. F. SHAPIRA and J. C. AVISE, 1983 The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. **80:** 1969–1971.

LESLIE, J. F., and R. C. VRIJENHOEK, 1978 Genetic dissection of clonally inherited genomes of *Poeciliopsis*. I. Linkage analysis and preliminary assessment of deleterious gene loads. Genetics **90:** 801–811.

LESLIE, J. F., and R. C. VRIJENHOEK, 1980 Consideration of Muller's ratchet mechanism through studies of genetic linkage and genomic compatibilities in clonally reproducing *Poeciliopsis*. Evolution **34:** 1105–1115.

MILLER, R. R., 1960 Four new species of viviparous fishes, genus *Poeciliopsis* from northwestern Mexico. Occas. Pap. Mus. Zool. Univ. Mich. **433:** 1–9.

MORITZ, C. C., T. E. DOWLING and W. M. BROWN, 1987 Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. **18:** 269–292.

MORITZ, C. C., J. W. WRIGHT and W. M. BROWN, 1989 Mitochondrial-DNA analyses and the origin and relative age of

parthenogenetic lizards (genus *Cnemidophorus*). III. *C. velox* and *C. exsanguis*. Evolution **43:** 958–968.

MORITZ, C., W. M. BROWN, L. D. DENSMORE, J. W. WRIGHT, D. VYAS, S. DONNELLAN, M. ADAMS and P. BAVERSTOCK, 1989 Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekonidae), pp. 87–112 in *Evolution and Ecology of Unisexual Vertebrates*, edited by R. DAWLEY and J. BOGART. New York State Museum, Albany, N.Y.

MULVEY, M., and R. C. VRIJENHOEK, 1981 Characterization of *Biomphalaria glabrata* strains by starch-gel electrophoresis. Biochem. Genet. **19:** 1169–1179.

NEI, M., and F. TAJIMA, 1981 DNA polymorphism detectable by restriction endonucleases. Genetics **97:** 145–163.

ROHLF, F. J., J. KISHPAUGH and D. KIRK, 1974 Numerical Taxonomy System of Multivariate Statistical Programs.

SCHENCK, R. A., and R. C. VRIJENHOEK, 1986 Spatial and temporal factors affecting coexistence among sexual and clonal forms of *Poeciliopsis*. Evolution **40:** 1060–1070.

SCHULTZ, R. J., 1969 Hybridization, unisexuality and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am. Nat. **103:** 605–619.

SCHULTZ, R. J., 1973a Origin and synthesis of a unisexual fish, pp. 207–211 in *Genetic and Mutagenesis of Fish*, edited by J. H. SCHOEDER. Springer-Verlag, New York.

SCHULTZ, R. J., 1973b Unisexual fish: laboratory synthesis of a "species." Science **179:** 180–181.

SCHULTZ, R. J., 1977 Evolution and ecology of unisexual fishes. Evol. Biol. **10:** 277–331.

SITES, J. W., JR., D. M. PECCININI-SEALE, C. MORITZ, J. W. WRIGHT and W. M. BROWN, 1990 The evolutionary history of parthenogenetic *Cnemidophorus lemniscatus* (Sauria: Teiidae). I. Evidence for a hybrid origin. Evolution **44:** 906–921.

SMOUSE, P. E., J. C. LONG and R. R. SOKAL, 1986 Multiple regression and correlation extensions of the Mantel Test of matrix correspondence. Syst. Zool. **35:** 627–632.

SPINELLA, D. G., and R. C. VRIJENHOEK, 1982 Genetic dissection of clonally inherited genomes of *Poeciliopsis*. II. Investigation of a silent carboxylesterase allele. Genetics **100:** 279–286.

SPOLSKY, C., and T. UZZELL, 1986 Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. Mol. Biol. Evol. **3:** 44–56.

VRIJENHOEK, R. C., 1975 Gene dosage in diploid and triploid unisexual fishes, pp. 463–475 in *Isozymes IV. Genetics and Evolution*, edited by C. L. MARKERT. Academic Press, New York.

VRIJENHOEK, R. C., 1979a Factors affecting clonal diversity and coexistence. Am. Zool. **19:** 787–797.

VRIJENHOEK, R. C., 1979b Genetics of a sexually reproducing fish in a highly fluctuating environment. Am. Nat. **113:** 17–29.

VRIJENHOEK, R. C., 1984 The evolution of clonal diversity in *Poeciliopsis*, pp. 399–429 in *Evolutionary Genetics of Fishes*, edited by B. J. TURNER. Plenum Press, New York.

VRIJENHOEK, R. C., 1989 Genetic and ecological constraints on the origins and establishment of unisexual vertebrates, pp. 24–31 in *Evolution and Ecology of Unisexual Vertebrates*, edited by R. DAWLEY and J. BOGART. New York State Museum, Albany, N.Y.

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.

VRIJENHOEK, R. C., R. A. ANGUS and R. J. SCHULTZ, 1978 Variation and clonal structure in a unisexual fish. Am. Nat. **112:** 41–55.

VRIJENHOEK, R. C., and R. J. SCHULTZ, 1974 Evolution of a trihybrid unisexual fish (*Poeciliopsis*, Poeciliidae). Evolution **28:** 205–319.

VRIJENHOEK, R. C., R. M. DAWLEY, C. J. COLE and J. P. BOGART, 1989 A list of known unisexual vertebrates, pp. 19–23 in *Evolution and Ecology of Unisexual Vertebrates*, edited by R, DAWLEY and J. BOGART. New York State Museum, Albany, N.Y.

VYAS, D. K., C. MORITZ, D. M. PECCININI-SEALE, J. W. WRIGHT and W. M. BROWN, 1990 The evolutionary history of parthenogenetic *Cnemidophorus lemniscatus* (Sauria: Teiidae). II. Maternal origin and age inferred from mitochondrial DNA analyses. Evolution **44:** 922–932.

WETHERINGTON, J. D., K. E. KOTORA and R. C. VRIJENHOEK, 1987 A test of the spontaneous heterosis hypothesis for unisexual vertebrates. Evolution **41:** 721–731.

WETHERINGTON, J. D., R. A. SCHENCK and R. C. VRIJENHOEK, 1989 Origins and ecological success of unisexual *Poeciliopsis*: the Frozen Niche Variation model, pp. 259–276 in *The Ecology and Evolution of Poeciliid Fishes*, edited by G. A. MEFFE and F. F. SNELSON, JR. Prentice-Hall, Englewood Cliffs, N.J.

WETHERINGTON, J. D., S. C. WEEKS, K. E. KOTORA and R. C. VRIJENHOEK, 1989 Genotypic and environmental components of variation in growth and reproduction of fish hemiclones (*Poeciliopsis*: Poeciliidae). Evolution **43:** 635–645.

Communicating editor: J. R. POWELL