Diversity in the Reproductive Modes of Females of the *Rutilus alburnoides* Complex (Teleostei, Cyprinidae): A Way to Avoid the Genetic Constraints of Uniparentalism

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Hybrid minnows collectively known as the *Rutilus alburnoides* complex are found throughout much of the Iberian Peninsula and include diploid and polyploid forms with female-skewed sex ratios. Previous studies have suggested that diploid and triploid females from the northern Douro Basin reproduce by hybridogenesis. The present study, which is based on experimental crosses and uses allozyme and minisatellite markers, reveals that diploid females from the Tejo Basin exhibit a different form of reproduction, transmitting the hybrid genome intact to the egg, which, upon fertilization, yields triploid progeny. Reproduction by triploid females from the southern Guadiana and Tejo basins resembles hybridogenesis in that one genome is discarded in each generation without recombination, but the remaining two homospecific genomes are not transmitted clonally. Elimination of the unmatched genome permits ready synapsis and meiosis between the homospecific genomes, and genetically distinct haploid eggs are produced (''meiotic hybridogenesis''). In some females, some sexual cells undergo an altered nonreductional meiosis, resulting in genetically diverse diploid eggs. In contrast to most hybrid vertebrate complexes, in which diploids and triploids are evolutionarily independent, in the *R. alburnoides* complex, there is a bidirectional movement of genes between diploid and triploid hybrids. Reproduction by the types of diploid and triploid females discussed here introduces high genotypic diversity into hybrid populations, and allows purging of deleterious genes and incorporation of beneficial mutations in the same genome, characteristics believed to be major advantages of sexual reproduction.

Introduction

There are only a few exceptions among vertebrates to the universal manner in which genetic information is transmitted between generations. These exceptions to sexual reproduction seem to be linked with hybridization: the combination of two heterospecific genomes from certain pairs of species skews the sex ratio in the hybrids toward females and alters gametogenesis such that the hybrid females produce eggs without recombination, founding uniparental lineages (reviewed in Dawley 1989). Three reproductive modes are recognized among the uniparental vertebrates whose reproductive mechanisms are understood: (1) parthenogenesis, in which the hybrid genome is transmitted intact to the eggs, which develop into genetically identical offspring in the absence of sperm; (2) gynogenesis, in which the process is the same as above, but sperm from a related species is required to stimulate embryogenesis; and (3) hybridogenesis, in which the part of the hybrid genome derived from one parental species is transmitted to the egg without recombination, while the genome from the other parental species is discarded and replaced in each generation through fertilization. The former two processes are clonal, whereas the latter is hemiclonal (one genome, usually the maternal genome, is inherited clonally, and the other, the paternal genome, is inherited sexually) (Dawley 1989).

Key words: *Rutilus alburnoides* complex, hybrid vertebrates, uniparental reproduction, modified hybridogenesis, crossing experiments.

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The absence of genetic recombination constitutes an evolutionary constraint on uniparental organisms. Lack of recombination precludes adjustment to temporal environmental changes, the incorporation in the same genome of beneficial mutations that arise in different individuals, and the removal of deleterious ones, compromising long-term survival (see Michod and Levin 1988 for review, in particular chapters 4, 7, 9, and 15). Recently, however, several studies have posed challenges to the traditional assumption that genomes of the uniparental vertebrates are inherited en bloc, as low levels of recombination have been suggested for some uniparental amphibians (Bogart 1989; Graf and Polls Pelaz 1989) and reptiles (Parker, Walker, and Paulissen 1989; Sites et al. 1990), and incorporation of subgenomic amounts of DNA from a bisexual host in a gynogenetic fish has been described (Schartl et al. 1995). Extending these studies to a broader taxonomic spectrum of uniparental vertebrates will probably reveal other evolutionary mechanisms that may compensate for the disadvantages of uniparentalism.

The ''*Rutilus* (a.k.a. *Tropidophoxinellus*) *alburnoides* (Steindachner 1866) complex'' is found throughout much of the Iberian Peninsula of southwest Europe. It includes diploid, triploid, and tetraploid females and males, with triploid females predominating in almost all populations (Collares-Pereira 1985, 1989; Alves, Coelho, and Collares-Pereira 1997; Carmona et al. 1997; Martins et al. 1998). Protein electrophoresis revealed that all females and most males of all ploidy levels exhibit nearly fixed heterozygosity at numerous allozyme loci, suggesting that they arose by interspecific hybridization (Alves, Coelho, and Collares-Pereira 1997; Carmona et al. 1997). One haploid genome was indistinguishable from that of sympatric populations of the ge-

nus *Leuciscus, L. carolitertii* in the more northern basins and *L. pyrenaicus* in the southern ones, while a second genome could not be attributed to any known biparental species. Alves, Coelho, and Collares-Pereira (1997) and Carmona et al. (1997) collected a low number of diploid specimens from the southern Tejo and Guadiana basins which contrasted with the remaining *R. alburnoides,* as they were homozygous at diagnostic loci for alleles not found in *L. pyrenaicus* (following the terminology of Carmona et al. (1997), these specimens are hereafter referred to as ''nonhybrids''). Carmona et al. (1997), taking into account that Hardy-Weinberg analysis of the polymorphic loci of the nonhybrid specimens revealed no significant deviations from random expectations, suggested that the complex comprises sexually reproducing diploid individuals. However, we think that this hypothesis requires further support, as the genotypic proportions in a small sample like that used by Carmona et al. (1997) are rarely significantly different from the Hardy-Weinberg expectations (Lewontin and Cockerham 1959). Moreover, the sex ratio of this form is strongly skewed toward males, as nonhybrid females are very rare (Carmona et al. 1997) or absent (unpublished data) in natural populations.

Assessment of mitochondrial (mt) DNA variation indicated a monophyletic relationship between the *R. alburnoides* hybrids, the nonhybrid specimens, and *L. pyrenaicus* (Alves et al. 1997; Carmona et al. 1997; unpublished data). This result was considered by Carmona et al. (1997) as evidence that the nonhybrid form is the maternal ancestor of the hybrid complex, leaving the *Leuciscus* ancestor as the paternal parent. According to this hypothesis, the similarity between the mtDNA of *L. pyrenaicus* and that of *R. alburnoides* is the result of introgression from *R. alburnoides* into *L. pyrenaicus.* However, this suggestion was based on the analysis of specimens of *L. pyrenaicus* collected from a single locality of the Guadiana drainage. The analysis of specimens from several basins (Alves et al. 1997) revealed that *R. alburnoides* is a polyphyletic lineage within *L. pyrenaicus* throughout its range, requiring widespread directional introgression. Placing the data from Carmona et al. (1997) into a larger framework, *R. alburnoides* most likely resulted from multiple nonreciprocal hybridizations involving females of *L. pyrenaicus.* According to this model, the nonhybrid specimens were most likely reconstituted from the hybrids, and their nuclear genome is probably very similar to that of the putative paternal ancestor of *R. alburnoides.*

The mechanisms by which the different *R. alburnoides* hybrids are perpetuated in nature are not yet well understood. Preliminary results obtained with allozyme markers revealed that triploid females from the Tejo Basin mated to *L. pyrenaicus* males produce diploid and triploid progeny which show evidence of sperm incorporation. This suggests that triploid females from the Tejo Basin may reproduce by hybridogenesis (Alves, Coelho, and Collares-Pereira 1996). Carmona et al. (1997), using allozyme patterns of mature primary oocytes, confirmed this reproductive mode for both diploid and triploid females from the northern Douro Basin, and verified that the *L. carolitertii* genome is discarded during oogenesis. Here, we describe an in-depth genetic analysis of the progeny from diploid and triploid females from the Tejo and Guadiana basins, using allozyme and minisatellite markers. These females were experimentally crossed with nonhybrid and *L. pyrenaicus* males. Minisatellites are highly variable noncoding regions of nuclear DNA which comprise short tandem repeat sequences differing in number of repetitions (Jeffreys, Wilson, and Thein 1985*a,* 1985*b*). Restriction fragment profiles generated by hybridization of hypervariable minisatellite probes are often unique to an individual and provide a powerful tool for investigating paternal genetic contribution and low levels of recombination in uniparental reproduction. The reproductive mode(s) of the hybrid males will be discussed elsewhere.

Materials and Methods

Breeding Experiments

Specimens used in the breeding experiments were collected during the reproductive season (April–May) from 1994 through 1996 from two sites: the Sorraia River of the Tejo Basin, and the Degebe River of the Guadiana Basin (detailed locality data are available from M.J.C.P.). In the Sorraia River, the *R. alburnoides* complex includes diploid (0%–15%), triploid (50%–100%), and tetraploid (0%–8%) hybrid females; diploid (0%–23%), triploid (0%–11%), and tetraploid (0%–14%) hybrid males; and nonhybrid diploid males (0%–19%). In the Degebe River, mainly triploid hybrid females (11%–88%), nonhybrid males (8%–89%), and, more rarely, diploid female (0%–11%) and triploid male (0%–5%) hybrids are found (Alves, Coelho, and Collares-Pereira 1997; Alves et al. 1997; Martins et al. 1998; unpublished data). Mates were randomly chosen, as crosses were done blindly without knowledge of the ploidy of specimens. Here, we present results of the crosses that involved diploid and triploid hybrid females and nonhybrid and *L. pyrenaicus* males.

Specimens were transported to the laboratory, and ripe females were stripped and their eggs exposed to sperm. The resulting progeny were reared to the age of 9 months (2–4 cm). High mortality occurred in the broods of 1996 due to fungal contamination. Parents and offspring were killed with an overdose of MS222 and frozen at −80°C. Offspring were sexed by dissection and inspection of gonads.

Ploidy Determination

Ploidy of parents and offspring was determined by flow cytometric measurement of erythrocyte DNA content as described in Dawley and Goddard (1988). Blood samples were drawn from the caudal vein, stabilized in buffer (40 mM citric acid trisodium salt, 0.25 M sacarose, and 5% DMSO), and immediately frozen at −80°C.

Multilocus DNA Fingerprinting

DNA from parents and offspring was extracted from blood and/or muscle following the standard SDS-

proteinase K/phenol-chloroform procedure for total genomic DNA extraction (Hillis et al. 1996).

Five restriction enzymes (*Alu*I, *Hae*III, *Hin*fI, *Mbo*I, and *Msp*I) were tested in combination with the human minisatellite probes 33.6 and 33.15 (Jeffreys, Wilson, and Thein 1985*a*) using DNA from four unrelated individuals. The best scorable band patterns were obtained with the restriction enzyme *Mbo*I. Five micrograms of DNA from parents and offspring was digested overnight using 10 U of this enzyme and following the conditions recommended by the supplier (Amersham). The DNA digest was separated on a 0.8% agarose gel (20 cm long, run at 40 V for 40 h). Families were run in parallel on the same electrophoretic gel. DNA was then depurinated and denatured in situ before capillary transfer onto nylon membranes (Hybond-Nfp, Amersham) as described in Bruford et al. (1992). DNA was fixed to membranes with UV irradiation on a 312-nm transilluminator. Probes were labeled with ^{32}P using the Multiprime DNA Labeling Systems kit (Amersham). Membranes were hybridized and washed under the conditions recommended by Bruford et al. (1992). Autoradiographs were produced by 1–7 days exposure with one intensifying screen. Hybridization to probe 33.15 was carried out after removal (confirmed by autoradiography) of all previously hybridized 33.6 by incubating membranes in 0.4 M NaOH at 45°C for 30 min, and in 0.2 M Tris-HCl (pH 7.5), 0.1 × SSC, 0.1% SDS at the same temperature for 15 min.

For each family, DNA patterns were scored manually from the original autoradiographs taken at short and long exposures, and the presence or absence of bands of similar electrophoretic mobility was recorded. The segregation pattern of the parental bands was analyzed to test the hypothesis of Mendelian inheritance. The number of offspring receiving each fragment was compared with the expected number given by the binomial distribution, in which the proportion of parental fragments that are transmitted to precisely r offspring in a shibship of N is $^{N}Cr/2^{N}$ (Jeffreys et al. 1986), using the G-test for goodness of fit (Sokal and Rohlf 1981).

Electrophoresis of Diagnostic Allozymes

Parents and offspring were examined by horizontal starch electrophoresis at the loci *sAAT** (aspartate aminotransferase, EC 2.6.1.1) and *PGDH** (phosphogluconate dehydrogenase, EC 1.1.1.44). These loci constitute good genetic markers for segregation analysis, as virtually all specimens of *R. alburnoides* hybrids express heterozygous patterns (Alves, Coelho, and Collares-Pereira 1997; Carmona et al. 1997). Parents from the Degebe River and their offspring were additionally analyzed at the locus *MDH-A** (NAD-dependent malate dehydrogenase, EC 1.1.1.37), as this locus has also proved to be a good genetic marker in the Guadiana Basin (Alves, Coelho, and Collares-Pereira 1997).

Results

Of the 15 *R. alburnoides* females bred in this study, 2 were diploid and 13 were triploid. Both diploid females were collected from the Sorraia River and mated to nonhybrid males. Triploid females were collected from the Sorraia and Degebe rivers and crossed with both *L. pyrenaicus* and nonhybrid males.

Table 1
Laboratory Crosses

Cross	Year	Parents' Origin	Ploidy	Analyzed Progeny: Sex ♀	Sex ♂	Sex ?
2n hybrid ♀ × nonhybrid ♂						
131	1996[a]	Sorraia	3n	5	—	—
138	1996[a]	Sorraia	3n	6	—	—
3n hybrid ♀ × *Leuciscus pyrenaicus* ♂						
2	1994	Sorraia	2n	15	8	7
3	1994	Sorraia	2n	1	17	12
9	1994	Sorraia	2n	7	2	7
			3n	5	—	7
58	1995	Degebe	2n	35	—	15
62	1995	Degebe	2n	25	—	2
67	1995	Degebe	2n	13	—	—
90	1996[a]	Degebe	2n	8	1	—
95	1996[a]	Degebe	2n	7	—	—
			3n	4	1	2
3n hybrid ♀ × nonhybrid ♂						
56	1995	Degebe	2n	—	13	6
64	1995	Degebe	2n	—	31	19
88	1996[a]	Degebe	2n	—	1	—
			3n	—	5	6
99	1996[a]	Degebe	2n	—	1	1
104	1996[a]	Sorraia	2n	—	2	—

[a] High mortality occurred in the broods of 1996 due to fungal contamination.

Diploid Females Mated to Nonhybrid Males

Diploid females crossed with nonhybrid males (crosses 131 and 138) yielded all triploid and female progeny (table 1). The analysis of the DNA fingerprints of both families (fig. 1*a* and appendix) revealed that all siblings displayed bands that could be traced back to the male parent, suggesting that sperm had been incorporated. The observed distribution of the paternal bands in the offspring followed the expected binomial distribution for alleles showing Mendelian inheritance ($P > 0.25$). All scorable maternal DNA fragments were coinherited by all progeny, indicating that the diploid female hybrid genome was transmitted intact to the eggs. One maternal fragment detected by probe 33.15 in cross 131 was an exception to this rule, as one descendant did not exhibit it.

Triploid Females Mated to *L. pyrenaicus* or Nonhybrid Males

Six triploid females bred with males of *L. pyrenaicus* produced broods of diploid progeny (crosses 2, 3, 58, 62, 67, and 90), and two produced mixed broods of diploid and triploid progeny in roughly equal numbers (crosses 9 and 95) (table 1). Most progeny of females from the Degebe River were female, except for one diploid male and one triploid male from crosses 90 and 95, respectively. Females from the Sorraia River produced both females and males: female offspring dominated in

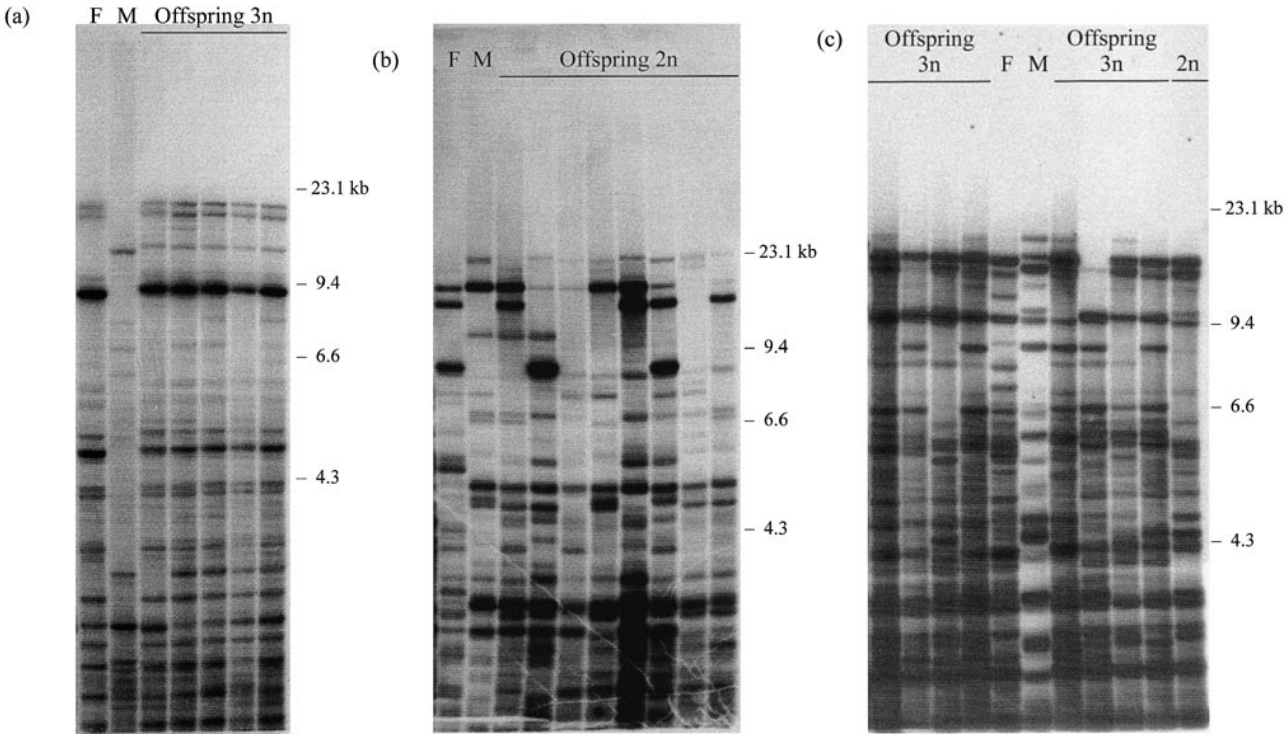

FIG. 1.—DNA fingerprints of parental fish and offspring of crosses 131 (*a*), 56 (*b*), and 88 (*c*), as obtained with probe 33.6 after *Mbo*I digestion.

crosses 2 and 9, while most progeny were males in cross 3.

Triploid females mated to nonhybrid males (crosses 56, 64, 88, 99, and 104) produced all male progeny. All broods were diploid, except for brood 88, for which all but one offspring were triploid.

Six families were analyzed by DNA fingerprinting: families 56, 58, 62 and 64, whose offspring were all diploid, and families 88 and 95, whose offspring were diploid and triploid (fig. 1*b* and *c*; appendix). Again, all offspring exhibited bands associated with the male parent, suggesting that the females produced reduced eggs which were fertilized. Inheritance of paternal bands of both *L. pyrenaicus* and nonhybrid males was Mendelian in that it followed the expected binomial distribution ($P > 0.1$). In each cross, a set of three to six maternal bands was transmitted to no offspring. This observation may be evidence for exclusion of one maternal genome, as the proportion of noninherited bands did not deviate significantly from the expected one third ($P > 0.05$). The remaining maternal fragments segregated in both diploid and triploid offspring. Maternal bands were inherited by the diploid offspring following the binomial distribution for alleles segregating in Mendelian fashion ($P > 0.1$). If the triploid young were formed by polyspermic fertilization of haploid eggs, the observed distribution of the maternal bands in the triploid offspring should also follow the expected binomial distribution. This hypothesis was rejected for both cross 88 and cross 95 ($P < 0.001$). Therefore, some triploid females produced two populations of genetically distinct eggs of different ploidy levels.

Protein electrophoresis provided clues about which genome might have been excluded during oogenesis (table 2). Diploid and triploid offspring fathered by nonhybrid males did not express electromorphs characteristic of *L. pyrenaicus* at the loci *sAAT**, *MDH-A**, and *PGDH** (the **a* allozymes), although their mothers were clearly heterozygous at these loci. On the other hand, all diploid offspring fathered by *L. pyrenaicus* were heterozygous at the diagnostic loci. These data suggest that haploid and diploid eggs produced by triploid females did not carry the genome of *L. pyrenaicus*, but only, or practically only, genes of the other ancestor.

Discussion

Production of Triploid Offspring by Diploid Females

DNA fingerprinting of progeny mothered by diploids from the Tejo Basin demonstrated that each inherited the hybrid genome of its mother, plus an additional haploid component from its father. Therefore, diploid females must have produced unreduced eggs that yielded triploid progeny upon fertilization (fig. 2*a*).

Production of unreduced eggs by diploid *R. alburnoides* is comparable to the mechanisms that operate in parthenogenetic and gynogenetic vertebrates. In such uniparental vertebrates, however, most eggs develop directly without syngamy into diploid clones, and only rarely is sperm incorporated to yield triploids (Dawley 1989). *Phoxinus eos-neogaeus* is apparently an exception, as it shows levels of syngamy of about 50% (Goddard and Dawley 1990). The number of offspring analyzed here is low, but the analysis suggests that syngamy

Table 2
Genotypes at Three Allozyme Markers in the Experimental Crosses

Cross		*sAAT**	*PGDH**	*MDH-A**	Total Examined
131.....	2n hybrid ♀	*a/c*	*a/c*	—	
	Nonhybrid ♂	*c/c*	*c/c*	—	
	Offspring	*a/c/–*	*a/c/–*	—	5
138.....	2n hybrid ♀	*a/c*	*a/c*	—	
	Nonhybrid ♂	*c/c*	*c/c*	—	
	Offspring	*a/c/–*	*a/c/–*	—	6
58.....	3n hybrid ♀	*a/c/–*	*a/c/–*	*a/b/b*	
	Leuciscus pyrenaicus ♂	*a/a*	*a/a*	*a/a*	
	Offspring	*a/c*	*a/c*	*a/b*	10
62.....	3n hybrid ♀	*a/c/–*	*a/c/–*	*a/b/b*	
	L. pyrenaicus ♂	*a/a*	*a/a*	*a/a*	
	Offspring	*a/c*	*a/c*	*a/b*	10
95.....	3n hybrid ♀	*a/c/–*	*a/c/–*	*a/b/b*	
	L. pyrenaicus ♂	*a/a*	*a/a*	*a/a*	
	Offspring	*a/c, a/c/–*	*a/c, a/c/–*	*a/b, a/b/b*	9
56.....	3n hybrid ♀	*a/c/–*	*a/c/–*	*a/b/b*	
	Nonhybrid ♂	*c/c*	*c/c*	*b/b*	
	Offspring	*c/c*	*c/c*	*b/b*	10
64.....	3n hybrid ♀	*a/c/–*	*a/c/–*	*a/b/b*	
	Nonhybrid ♂	*c/c*	*c/c*	*b/b*	
	Offspring	*c/c*	*c/c*	*b/b*	14
88.....	3n hybrid ♀	*a/c/–*	*a/c/–*	*a/b/b*	
	Nonhybrid ♂	*c/c*	*c/c*	*b/b*	
	Offspring	*c/c, c/c/c*	*c/c, c/c/c*	*b/b, b/b/b*	12

NOTE.—At the loci *sAAT** and *PGDH**, it was not possible to identify the allele dosage for the heterozygous triploids; the unknown allele is indicated by "–".

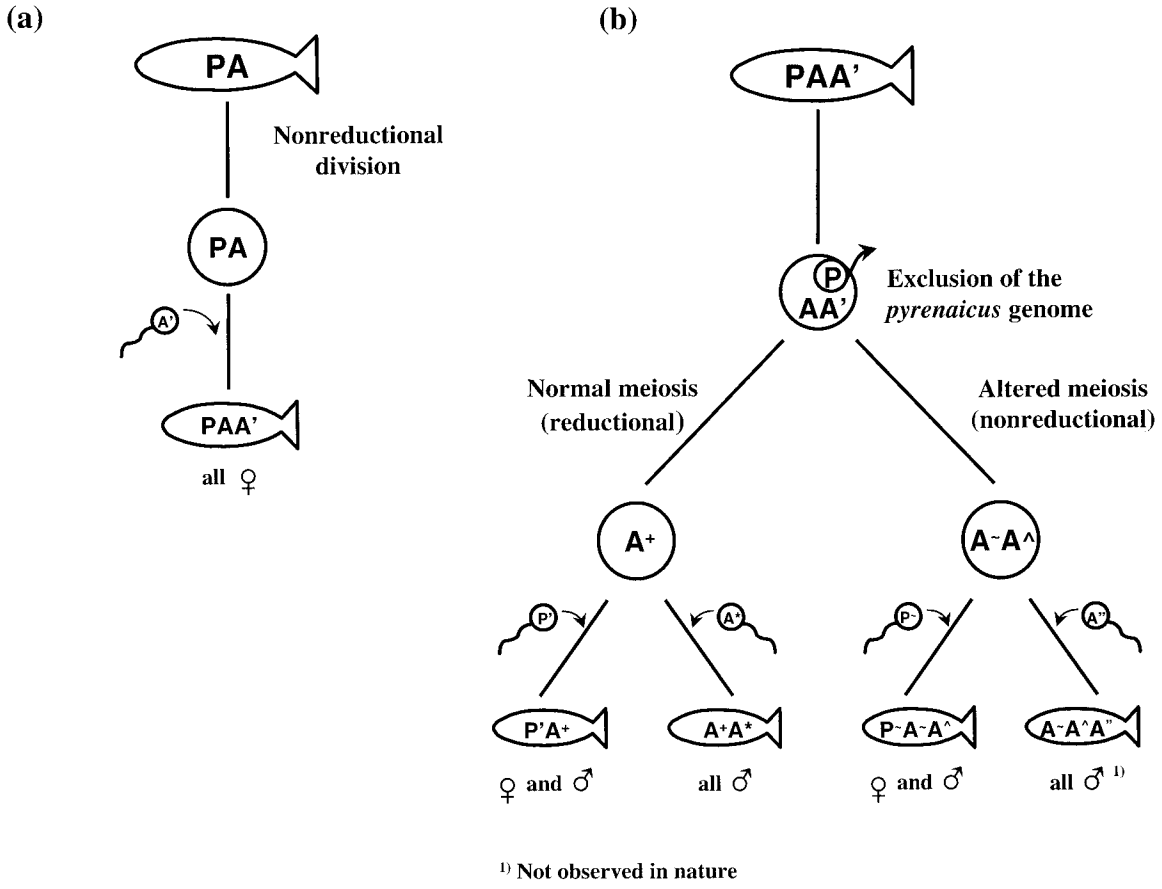

FIG. 2.—Modes of reproduction by *R. alburnoides* diploid females from the Tejo Basin (*a*) and triploid females from the Guadiana and Tejo basins (*b*), as inferred from the crossing experiments. P is the genome of *L. pyrenaicus*, and A is the genome of the other ancestor. The prime signs indicate that the various P and A genomes are not identical.

occurs almost always, rather than occasionally, in diploid eggs produced by diploid *R. alburnoides.*

One maternal fragment in female 131 was not transmitted to all offspring. This variation is probably due to germ line or somatic mutation which produced a new length allele (Jeffreys et al. 1988). Evidence for genomic variability in as short a time as one generation has also been provided for the gynogenetic *Poecilia formosa* (Monaco, Rasch, and Musich 1988; Schartl et al. 1990, 1991).

The data presented here contrast with the results of Carmona et al. (1997) for diploid hybrid females from the Douro Basin. The authors analyzed allozyme patterns of mature primary oocytes and concluded that the diploid females discarded the *L. carolitertii* genome during oogenesis. Different hybrid backgrounds seem to disrupt oogenesis differently, as diploid female hybrids from the Douro Basin possess a nuclear genome from *L. carolitertii,* whereas those from the Tejo Basin contain a genome from *L. pyrenaicus* (Alves, Coelho, and Collares- Pereira 1997; Carmona et al. 1997). A similar phenomenon has been reported by Hotz and Uzzell (1983) and Hotz et al. (1985) for *Rana esculenta.*

Production of Diploid and Triploid Progeny by Triploid Females

The characteristics of the offspring mothered by triploid *R. alburnoides* from the Tejo and Guadiana basins suggest that they produced haploid and, more rarely, diploid gametes, which were fertilized. The genome of *L. pyrenaicus* was excluded during oogenesis, probably by a mechanism similar to those which operate in hybridogenetic vertebrates (Dawley 1989). These results are consistent with the results of Carmona et al. (1997), who found that triploid females from the Douro Basin produced reduced oocytes that expressed no alleles from *L. carolitertii.*

However, although reproduction by triploid females resembles hybridogenesis, all offspring showed evidence of segregation of the nonexcluded maternal genomes, suggesting that in the mechanism of maturation of the haploid and diploid eggs, at least part of normal meiosis was retained (''meiotic hybridogenesis''). The present data are consistent with the hypothesis of Günther, Uzzell, and Berger (1979), who suggested that the presence of two homospecific genomes in allotriploids should permit ready synapsis and normal meiosis after the elimination of the unmatched genome. Production of haploid eggs has been reported for other triploid hybrid vertebrates (Nishioka and Ohtani 1984; Goddard and Schultz 1993), but segregation of the homospecific genomes was clearly demonstrated only for triploid *R. esculenta* (Günther, Uzzell, and Berger 1979). In three triploid females, some sexual cells did not undergo normal meiosis, producing diploid eggs (as much as 43%–92%). The ploidy level, together with the fact that sibships are heterogenic, suggests that those cells suppressed division I or II of meiosis or that there was a reentry of the second polar body and crossing over between homologous chromosomes occurred (Uzzell 1970).

Normal Meiosis in Nonhybrid Males

The nonhybrid males used in the present study exhibited Mendelian segregation at the minisatellite markers. This observation is consistent either with the hypothesis that the *R. alburnoides* comprises sexually reproducing diploid individuals (Carmona et al. 1997) or with the alternative hypothesis that these specimens have been regenerated from the hybrids. As demonstrated by Leslie and Vrijenhoek (1978, 1980), reconstituted individuals are equally expected to recombine in a normal Mendelian meiosis, because they possess two homospecific genomes.

Breeding Dynamics

Inheritance patterns in crosses involving diploid and triploid female *R. alburnoides* collected from the Tejo and Guadiana basins provide some insight into the putative breeding dynamics of natural populations. In natural populations from those basins, ploidy elevation and reduction are probably common events and allow a bidirectional movement of genes between diploids and triploids: diploid females produce unreduced eggs that create triploid females upon fertilization; triploid females produce haploid and, more rarely, diploid eggs that give rise to diploids and triploids upon sperm incorporation (fig. 2). Among the known hybrid species complexes, the *R. alburnoides* complex seems most similar to those of *Ambystoma* and *Phoxinus* hybrids, in which ploidy shifts are common (Bogart and Licht 1986; Bogart 1989; Kraus 1989; Goddard and Dawley 1990; Goddard and Schultz 1993).

Depending on the male species, diploid females are expected to produce progeny with two genomes of *pyrenaicus* and one of the other ancestor (PPA), or with one genome of *pyrenaicus* and two of the other ancestor (PAA). Triploid PAA females produce A and/or AA eggs, which produce offspring with PA and PAA constitutions upon fertilization by P sperm from a male *L. pyrenaicus,* and yield AA or AAA males upon fertilization by a A sperm from a nonhybrid male. Individuals with AAA genomic composition have never been observed in nature (Alves, Coelho, and Collares-Pereira 1997; Carmona et al. 1997). Although it was not experimentally tested, we may expect that PPA hybrids also discard the unmatched genome, producing P and/or PP gametes. If these eggs are fertilized by A sperm, they will originate PA and PPA offspring, and if they are fertilized by P sperm, they will produce PP and PPP offspring. PP individuals with a hybrid ancestor will be indistinguishable from sexually produced *L. pyrenaicus.* A triploid specimen identified as *L. pyrenaicus* on the basis of its morphology was collected at the Sado Basin (Collares-Pereira 1983). If some genetic recombination between the heterospecific genomes occurs in the triploid hybrids, PP offspring may be a vehicle for introgression of A alleles into *L. pyrenaicus.* Evidence for interspecific gene flow mediated by uniparental hybrid populations has been reported by Uzzell, Günther, and Berger (1977), Uzzell (1982), and Bogart (1989).

Although diploids may be abundant in some localities (for example, diploid males constituted 89% of the

total of specimens collected from one sampling site in the Degebe River; Martins et al. 1998), triploid females predominate throughout the Guadiana and Tejo basins. The breeding experiments presented here indicate that most triploid females (10 out of 13) produced exclusively haploid eggs, which, upon fertilization, yielded diploid offspring. Consequently, a higher proportion of diploids would be expected under natural conditions. In order to assess the factors that act on the population structure of the *R. alburnoides* complex, future experiments are required to (1) determine which factors affect ploidy of progeny, (2) investigate the fecundity patterns of diploid and triploid females, and (3) evaluate the survival of offspring of different genotypes under various environmental conditions. Some insight into possible factors that influence ploidy of progeny may come from artificial gynogenesis, where temperature shocks have long been employed to induce diploidization of oocytes (cf. Cherfas 1981).

Progeny Sex Ratio

The cytological data presented by Collares-Pereira et al. (1998) indicate that *L. pyrenaicus* has a ZW female/ZZ male sex chromosome heteromorphism. The sex ratio of the triploid offspring mothered by diploid hybrids (crosses 131 and 138) is in agreement with this model: having received Z and W chromosomes from their mothers, all progeny were female. However, a simple ZW/ZZ sex-determining mechanism does not explain why the sex ratio of progeny mothered by triploid females seems to be connected with the type of genome received from the father; if they receive an A genome from nonhybrid males, they produce only male progeny, whereas if they receive a P genome from *L. pyrenaicus,* they produce predominantly females or mixed broods of females and males. These results are in agreement with what we have observed in natural populations, where no AA females have been found (Alves, Coelho, and Collares-Pereira 1997; unpublished data). The offspring of cross 3 was exceptional in that they received a P genome and were essentially all male (1:17). Such a sex-determining mechanism does not explain why matings between triploid females and *L. pyrenaicus* males from the Degebe River yielded essentially all female offspring, whereas the same mating involving fishes from the Sorraia River produced both females and males. Again, these results agree with those we have observed in nature, namely that in the Degebe River, PA or PAA individuals are essentially all female, whereas in the Sorraia River, they include both females and males (Alves, Coelho, and Collares-Pereira 1997; unpublished data). In addition to female determinants on the W chromosome, a minimum of one non-W-linked gene, expressed differently in hybrid and nonhybrid genome combinations, has to be postulated to account for these observations. Sex-determining factors might vary in ''strength'' depending on the species and the population to which the parents belong. An analogous situation has been described in the hybridogenetic *Poeciliopsis monacha-lucida* (Schultz 1961, 1989) and *R. esculenta* (Berger, Uzzell, and Hotz 1988; Graf and Polls Pelaz 1989).

Conclusions

The reproductive modes of diploid and triploid female *R. alburnoides* from the Tejo and Guadiana basins cannot be conveniently placed into the three categories generally recognized for uniparental vertebrates (Dawley 1989). Like the gynogenetic vertebrates, diploid females clonally transmit their hybrid genomes, but sperm is apparently incorporated and expressed in all offspring. Triploid females present a modified hybridogenesis (''meiotic hybridogenesis'') in which one genome is discarded in each generation without recombination, but inheritance is not hemiclonal. Meiosis involves random segregation and recombination between the homospecific genomes, and genetically distinct haploid and diploid eggs are produced. Moreover, unlike what happens in hybridogenesis, a sperm genome that is incorporated into the progeny may remain in the hybrid lineages longer than one generation: the sperm genome that is incorporated in haploid eggs will likely not be discarded in the next generation, but clonally transmitted by the diploid females to their diploid eggs.

The reproductive modes by the types of diploid and triploid females reported here introduce high genotypic diversity into hybrid populations. In addition to the genotypic diversity that results from paternal genome incorporation in each generation, *R. alburnoides* also possesses genotypic diversity resulting from the occurrence of meiosis in triploid females. High genetic variability may explain in part the ecological success of *R. alburnoides,* which is one of the most abundant and widespread minnows of central and southern Iberian freshwaters. Moreover, as discussed in Carmona et al. (1997), the incorporation and expression of paternal genes may benefit the hybrids by increasing the likelihood of hybrid matings and helping the hybrids adapt to local conditions for which the sexual-host species are already well adapted. In the *R. alburnoides* complex, there is a continual genetic exchange between diploid and triploid hybrids. This contrasts with most uniparental vertebrate complexes, for which diploids and triploids are evolutionarily independent (Dawley 1989).

The present study highlights the power of minisatellite markers to study how genetic information is transmitted in hybrid organisms. In fact, allozyme markers alone (table 2) would not allow one to detect that the homospecific genomes of triploid *R. alburnoides* females segregated in a Mendelian fashion and, thus, that meiosis occurred. This finding is significant, as recombination is believed to be a major advantage of sexual reproduction. Meiosis in triploid *R. alburnoides* females involving the homospecific genomes may allow purging of deleterious gene loads and incorporation of beneficial mutations in the same genome. This gametogenic mechanism may rescue uniparental lineages from mutational meltdown and challenges the idea that reproductive modes other than sexual reproduction are inevitably dead ends.

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APPENDIX

Segregation of Hypervariable Single Fragments Produced by the Minisatellite Probes 33.6 and 33.15 (Jeffreys et al. 1985*a*) in the Experimental Families

No. of Offspring (*r*)	Cross 131 Offspring 3n (*N* = 5)	Cross 138 Offspring 3n (*N* = 5)	Cross 58 Offspring 2n (*N* = 8)	Cross 62 Offspring 2n (*N* = 8)	Cross 95 Offspring 2n (*N* = 7)	Cross 95 Offspring 3n (*N* = 6)	Cross 56 Offspring 2n (*N* = 8)	Cross 64 Offspring 2n (*N* = 8)	Cross 88 Offspring 3n (*N* = 8)
Observed Number of Single Maternal Fragments Transmitted to *r* Offspring									
0	0	0	5	3	3	3	4	3	6
1	0	0	3	1	1	0	0	0	0
2	0	0	1	6	3	3	1	2	0
3	0	0	5	4	4	1	4	2	2
4	1	0	3	2	3	1	3	9	2
5	33	21	5	3	3	5	2	2	0
6			0	1	0	0	2	1	4
7			0	1	0		0	0	8
8			0	0			0	0	0
Total no. of bands (*n*)	34	21	22	21	17	17	16	19	22

	Cross 131 Offspring 3n (*N* = 5)	Cross 138 Offspring 3n (*N* = 5)	Cross 58 Offspring 2n (*N* = 8)	Cross 62 Offspring 2n (*N* = 8)	Cross 95 Offspring 2n + 3n (*N* = 13)	Cross 56 Offspring 2n (*N* = 8)	Cross 64 Offspring 2n (*N* = 8)	Cross 88 Offspring 2n + 3n (*N* = 9)
Observed Number of Single Paternal Fragments Transmitted to *r* Offspring								
0	1	2	0	0	0	0	0	0
1	1	3	2	0	0	0	1	2
2	4	3	2	2	0	3	1	0
3	6	6	3	3	1	3	1	2
4	5	3	1	3	4	3	6	5
5	0	0	2	3	1	2	3	3
6			3	2	5	1	2	5
7			1	2	4	2	0	2
8			0	0	3	0	0	0
9					0			0
10					2			
11					1			
12					1			
13					0			0
Total no. of bands (*n*)	17	17	14	15	22	14	19	22

NOTE:—Fragments transmitted to all offspring may be from homozygous loci and were ignored for all but the diploid females. Only one of each set of apparently linked or allelic fragments was retained. Linkage may result from the cutting of a single minisatellite allele at internal recognition sites, generating two or more fragments which are always coinherited; alternatively, two distinct minisatellite regions may be situated close together on a chromosome so that recombination between them occurs infrequently (Bruford et al. 1992). Bands codetected by both probes were not observed. *N* = number of offspring analyzed for each brood.

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