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# Extensive Interspecific Genetic Compatibility in the New World Killifish Genus *Cyprinodon*

BRUCE J. TURNER AND ROBERT K. LIU

Interspecific hybrids in the oviparous killifish genus *Cyprinodon* have been produced in the laboratory from 14 different combinations (and 4 reciprocals) among 9 species. Hybrids were reared from all interspecific combinations from which fertilized ova were obtained. For the most part they were approximately intermediate in the expression of divergent parental morphological features but not in reproductive behavior. Although the majority of the hybrids were fertile, a few cases of male sterility or absence were recorded. Bispecific hybrids were used to produce a few trispecific and one fertile tetraspecific hybrid. The ease with which morphologically and behaviorally divergent *Cyprinodon* species are hybridized in the laboratory suggests that the widely accepted correlation between hybridization potential and phylogenetic relationship may not hold for *Cyprinodon* as well some other genera (e.g., *Lepomis*, *Xiphophorus*) of highly interfertile yet morphologically distinctive fish species.

THIS paper reports the results of hybridization experiments with nine species of the killifish genus *Cyprinodon* (Cyprinodontidae). These experiments were conducted in conjunction with our ethological and biochemical studies of divergence within the genus (Liu, 1969; Turner, 1973, 1974). Although cyprinodontoid fishes have been the subjects of hybridization experiments since the early years of this century (Newman, 1908), attention has generally been focused on the genus *Fundulus* or on exotic species (see Schwartz, 1972); with the exception of a few crosses reported by Miller (1948) and Drewry (1967), and two reports of natural hybridization associated with sympatry caused by human interference (Miller, 1968; Stevenson and Buchanan, 1973), *Cyprinodon* has been ignored. Our data reveal that morphologically and behaviorally distinctive *Cyprinodon* species are interfertile to a remarkable extent and therefore raise questions concerning the commonly accepted relationship between interspecific hybridization ability and genetic relatedness.

The genus *Cyprinodon* as currently recognized (Fig. 1) consists of 21 species (and a number of undescribed forms) which can be arranged in 4 complexes or species groups based on morphological similarities (Miller in Liu, 1969). The genus is essentially Nearctic in distribution, but several members of the *C. variegatus* complex have colonized the Greater Antilles and one species, *C. dearborni*, occurs on the Venezuelan coast and the Nether-

lands Antilles. With the exception of an apparent species-flock in an interior Yucatan lake (Humphries, 1975) all known *Cyprinodon* species are allopatric. The general biology, distribution and ecology of this genus of markedly euryplastic, euryhaline and eurythermal species has been reviewed in detail by Liu (1969); other pertinent studies are those of Echelle et al. (1972), Echelle (1973) and Martin (1972). The morphological divergence of most nominal *Cyprinodon* species is well marked, and many of them show distinctive motor patterns in their reproductive behavior, but the genetic divergence of some of them as detected by gel electrophoresis is slight (Turner, 1974) and the chromosome complements of all species thus far surveyed are essentially indistinguishable (Fig. 2; Stevenson, 1975; Miller and Echelle, 1975).

## MATERIALS AND METHODS

Live material used for hybridization experiments was as follows: 1) *Cyprinodon atrorus*: Laguna San Pablo, Cuatro Ciénegas basin, Coahuila, Mexico. 2) *Cyprinodon bifasciatus*: Laguna El Mojarral, Cuatro Ciénegas basin, Coahuila, Mexico. 3) *Cyprinodon eximius*: Rio Florido near Jimenez, Chihuahua, Mexico. 4) *Cyprinodon macularius*: Salton Sea, Imperial County, California (hybrids with *C. nevadensis*, *C. variegatus*, *C. salinus*, *C. bifasciatus*); Quito-baquito Springs, Organ Pipe Cactus National Monument, Arizona (hybrids with *C. atrorus*, *C. eximius*). 5) *Cyprinodon nevadensis nevadensis*: Saratoga Springs, Death Valley National

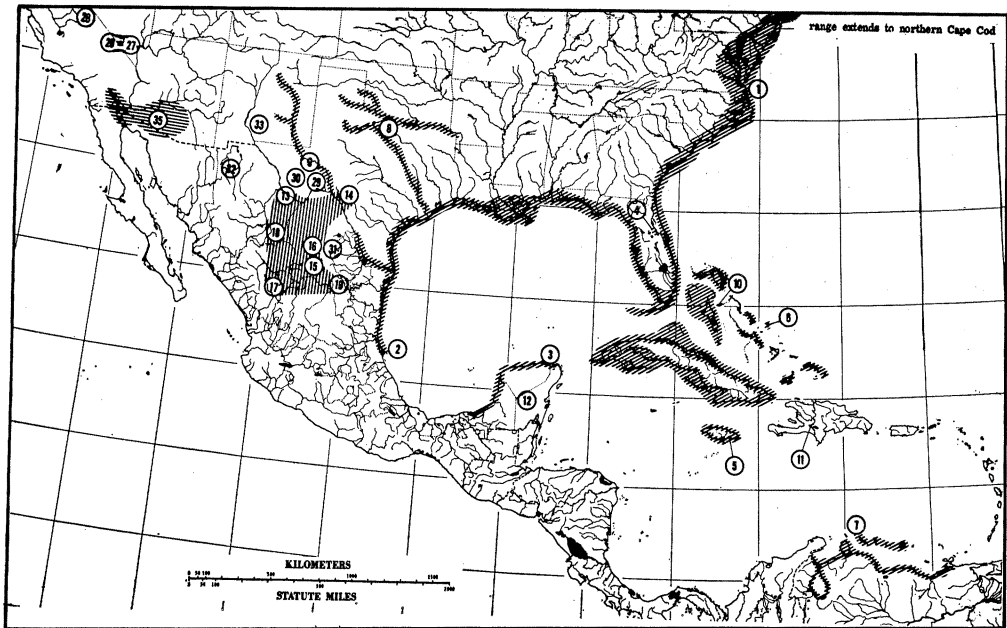


Fig. 1. Geographical distribution of the genus *Cyprinodon*; all known taxa are included. *C. variegatus* complex: 1. *C. variegatus ovinus* Mitchell. 2. *C. v. variegatus* Lacépède (range includes Cuba [formerly *C. v. riverendi*] and the Bahamas [formerly *C. baconi*]). 3. *C. v. artifrons* Hubbs. 4. *C. hubbsi* Carr. 5. *C. jamaicensis* Fowler (very doubtfully distinct from *C. v. variegatus*). 6. Differentiated lacustrine population or undescribed species (?), San Salvador. 7. *C. dearborni* Meek. 8. *C. rubrofluvialis* Fowler (range unsubstantiated east of Waco, Texas; also occurs in upper Colorado—Clark Hubbs, pers. comm.). 9. Undescribed species, Pecos River Basin. 10. *C. laciniatus* Hubbs and Miller. 11. *C. bondi* Myers. 12. *C. beltrani* Alvarez and undescribed sympatric congeners (Humphries, 1975). 33. *C. tularosa* Miller and Echelle. *C. eximius* complex: 13. *C. eximius* Girard. 14. Undescribed species, Devil's River, Texas (extinct). 15. *C. latifasciatus* Garman (extinct). 16. *C. atlorus* Miller. 17. Undescribed species, headwaters of the Rio Mezquital, Durango. 18. Undescribed species, near Jimenez, Chihuahua. 19. Undescribed species, Potosi, Nuevo Leon. *C. nevadensis* complex: 20–25. *C. nevadensis* Eigenmann and Eigenmann (6 subspecies, Miller, 1948). 26. *C. salinus* Miller and *C. milleri* LaBounty and Deacon. 27. *C. diabolis* Wales. *C. nevadensis*, *C. salinus*, *C. milleri*, and *C. diabolis* are not sympatric, but their localities are too proximate to be separately indicated on a map of this scale. 28. *C. radiosus* Miller. *C. macularius* complex: 35. *C. macularius* Baird and Girard (extant populations in the Salton Sea, Colorado delta, and Rio Sonoyta; these populations are somewhat morphologically divergent but have not been taxonomically differentiated). Misc. species: 29. *C. bovinus* Baird and Girard. 30. *C. elegans* Baird and Girard. 31. *C. bifasciatus* Miller. 32. Undescribed species, basins of the Rio Casas Grandes, Rio Sta. Maria, Rio del Carmen and Rio Papigochic, Chihuahua. Map is from Liu (1969) with minor revision; distribution of undescribed species (except no. 6) is from R. R. Miller (pers. comm.).

Monument, California. 6) *Cyprinodon nevadensis mionectes*: Big Spring, Ash Meadows, Nye County, Nevada. 7) *Cyprinodon radiosus*: Fish Slough, near Bishop, Mono County, California. 8) *Cyprinodon rubrofluvialis*(?): Bitter Lakes Wildlife Refuge, Chaves County, New Mexico. This population may be divergent from *C. rubrofluvialis* of the Red River Basin (Echelle, 1973). 9) *Cyprinodon salinus*: Salt Creek, Death Valley National Monument, California. 10) *Cyprinodon variegatus variegatus*: drainage ditch, vicinity of Miami, Florida. 11) *Cyprinodon variegatus ovinus*: Casey Pond, Nara-

gansett Bay, Rhode Island (hybrids with *C. macularius*); Westhampton Beach, Suffolk County, New York (hybrids with *C. nevadensis*); Hungerford Creek near Solomons, Maryland (testcross with female tetraspecific hybrid).

Hybrids were reared from fertilized ova collected from matings of single heterospecific pairs in aquaria; successful crosses were generally not replicated. Matings were often primarily intended for observations of reproductive behavior (Liu, 1969) and generally involved isolation of a single specimen of each sex for at least 24 hours prior to introduction of the

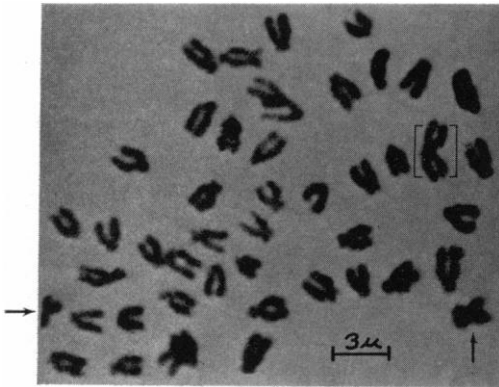


Fig. 2. Mitotic metaphase chromosomes of a male *Cyprinodon macularius* from Oasis, Salton Sea, California. Chromosome squash was prepared from pectoral fin epithelium by the method of McPhail and Jones (1966).  $2N = 48$ ; note the presence of 2 small metacentric to submetacentric elements (arrows). The brackets contain two acrocentric chromosomes with coincidentally overlapping centromeres. The chromosome complements of all *Cyprinodon* species thus far analyzed are essentially identical to the one shown here (compare with Miller and Echelle, 1975: Fig. 2).

female into an aquarium compartment occupied by the male. The advantages of this technique for behavioral studies have been discussed by Liu (1969:13) and Franck (1970). Previous isolation, however, is not a prerequisite for hybridization. With some matings (e.g., *C. nevadensis*  $\times$  *C. variegatus*) large quantities of ova were obtained a few days after directly transferring a male and female from their respective stock aquaria to a 10 l aquarium furnished with a 2 cm substrate of fine white sand. Ova were collected by directly sifting the sand or stirring it with a glass rod and rapidly swirling a small 1 mm mesh net in a figure-8 motion about 8 cm above the substrate. In some cases the spawning pair was removed from the aquarium and the ova allowed to develop and hatch in situ. In general, the laboratory production of *Cyprinodon* hybrids is no more difficult than the propagation of single species; specialized techniques like artificial fertilization appear unnecessary.

## RESULTS

Nine species were used to produce 18 interspecific hybrids, comprising 14 different combinations and 4 reciprocal crosses; 10 of the crosses were carried to the second or third generation. Bispecific hybrids are enumerated

in Table 1; we emphasize that a blank entry in the table indicates an unattempted, not an unsuccessful, cross. A  $F_1$  hybrid was produced in all crosses where fertilized ova were collected. We have no data on the relative fertility of heterospecific versus conspecific matings, but there was no readily apparent increase in mortality of hybrid zygotes.

In general shape and coloration  $F_1$  hybrids were approximately intermediate between parental extremes (Fig. 3). In most crosses, species-specific pigment patterns (terminal caudal bands in males, dorsal and/or anal fin ocelli in females) appeared to be dominant to their absence, although they often showed reduced expression. Reciprocal crosses of the same species (Fig. 3, e, f) produced hybrids that strongly resembled each other in shape and color pattern, suggesting minimal sex linkage of the genes encoding these traits in the parental species. Since  $F_2$  hybrids closely resembled their parents, there was no obvious assortment of parental traits.

In the following discussion the male parent of each hybrid cross is listed first:

**Behavior.**—The male courtship behavior of seven  $F_1$  hybrids and their parental species is summarized in Fig. 4. The behavioral motor patterns are classified in terms of male-specific displays, orientation positions and contact movements. Motor patterns involved in display behavior are "looping" ("L"), "looping around vertical axis" ("L<sub>a</sub>"), "looping circle" ("L<sub>c</sub>") and "circling, same plane as female" ("C<sub>s</sub>"). Orientation positions are "chasing below" ("C<sub>b</sub>"), and "chasing below position" ("C<sub>bp</sub>"). The four motor patterns involved in contact movements are three types of "nuzzling" ("nu<sub>1</sub>"—"nu<sub>3</sub>") and "nuzzling attempt during contacting attempt" ("nu/co"). These 10 motor patterns and their sequential relationships are discussed in detail by Liu (1969:54–59) and are shown in Fig. 4. Although these data are insufficient for detailed genetic analysis, several observations seem to be significant. The motor patterns involved in courtship are male-specific, yet hybrid males, in addition to paternal elements, often showed at least some motor patterns characteristic of males of the maternal species. The courtship repertoires of hybrid males were generally less than completely additive of those of the two parental species; a common or frequent paternal or maternal motor pattern often was absent or reduced in relative frequency. There was a clear-cut lack of resemblance between the reciprocal hybrids of *C.*

TABLE 1. GENERATIONS AND NUMBERS OF BISPECIFIC *Cyprinodon* HYBRIDS. ENTRIES IN THE MATRIX DENOTE THE NUMBER OF PROGENY REARED IN EACH HYBRID GENERATION.

Males:	<i>C. atrorus</i>	<i>C. bifasciatus</i>	<i>C. macularius</i>	<i>C. nevadensis</i>	<i>C. radiosus</i>	<i>C. rubro-fluviatilis</i>	<i>C. salinus</i>	<i>C. variegatus</i>
Females								
<i>C. atrorus</i>		F <sub>2</sub> <sup>3</sup>	F <sub>2</sub> >20; <5					X <sup>5,6</sup>
<i>C. eximius</i>	F <sub>2</sub> 5; <5		F <sub>2</sub> 15; <5					
<i>C. macularius</i>	F <sub>2</sub> 20; >5; <5	F <sub>2</sub> >5; <5		X <sup>6</sup>	X <sup>6</sup>		F <sub>1</sub> 5	F <sub>1</sub> >15 <sup>5</sup>
<i>C. nevadensis</i>	F <sub>2</sub> 5; <5	F <sub>1</sub> 5 <sup>1</sup>	F <sub>2</sub> 10 <sup>2,4</sup> ; >5		F <sub>2</sub> >5 <sup>2</sup> ; <5		F <sub>2</sub> 16; >40	F <sub>1</sub> 36 <sup>1,5</sup>
<i>C. salinus</i>				F <sub>1</sub> 12	X <sup>6</sup>	X <sup>6</sup>		
<i>C. variegatus</i>	X <sup>5,6</sup>	X <sup>5,6</sup>	F <sub>1</sub> 5 <sup>1,2</sup>	F <sub>1</sub> 32 <sup>1,5</sup>	X <sup>6</sup>	F <sub>1</sub> 5 <sup>2</sup>		

<sup>1</sup> Males absent, rare, or sterile; see text.<sup>2</sup> Hybrid was used in multispecific crosses; see text.<sup>3</sup> Hybrids obtained from W. I. Minckley, Arizona State University.<sup>4</sup> Cross replicated with *C. n. nevadensis* and *C. n. mionectes*.<sup>5</sup> The *C. variegatus* used in this cross were *C. v. ovinus*.<sup>6</sup> Successful mating observed but egg collection was not attempted.

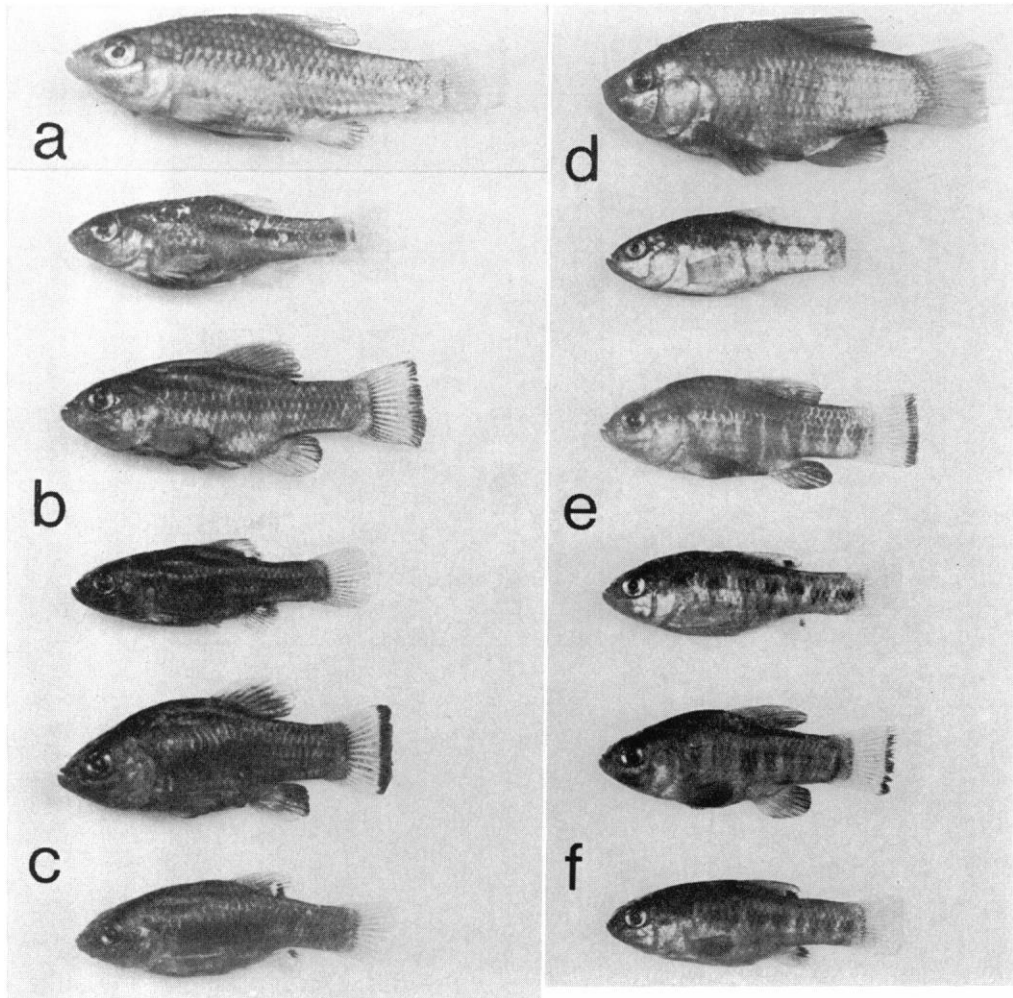


Fig. 3. Some  $F_1$  bispecific *Cyprinodon* hybrids and their parental species (males above, females below): a) *C. bifasciatus*. b)  $F_1$  *C. bifasciatus* male  $\times$  *C. atrorus* female. c) *C. atrorus*. d) *C. macularius*. e)  $F_1$  *C. atrorus* male  $\times$  *C. macularius* female. f)  $F_1$  *C. macularius* male  $\times$  *C. atrorus* female. Note general similarity of reciprocals in "e" and "f." Standard lengths of these specimens range from 26 to 40 mm.

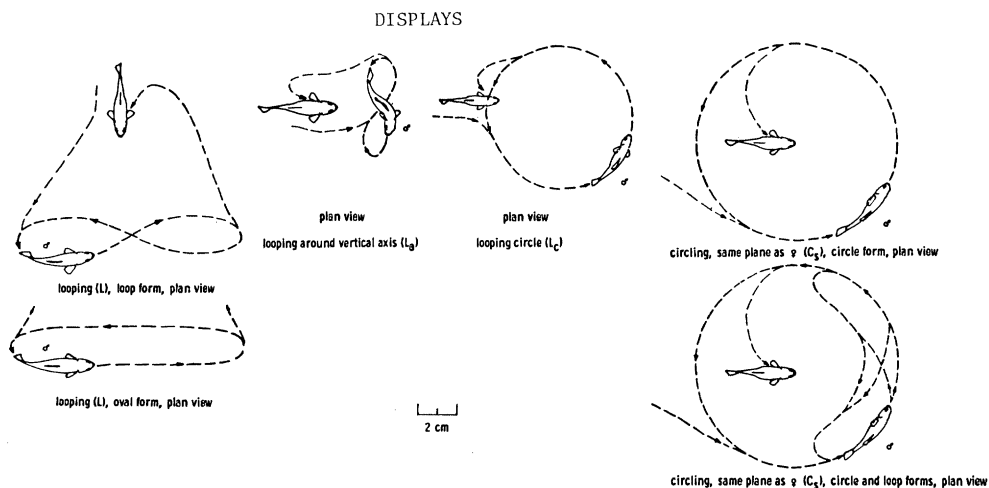
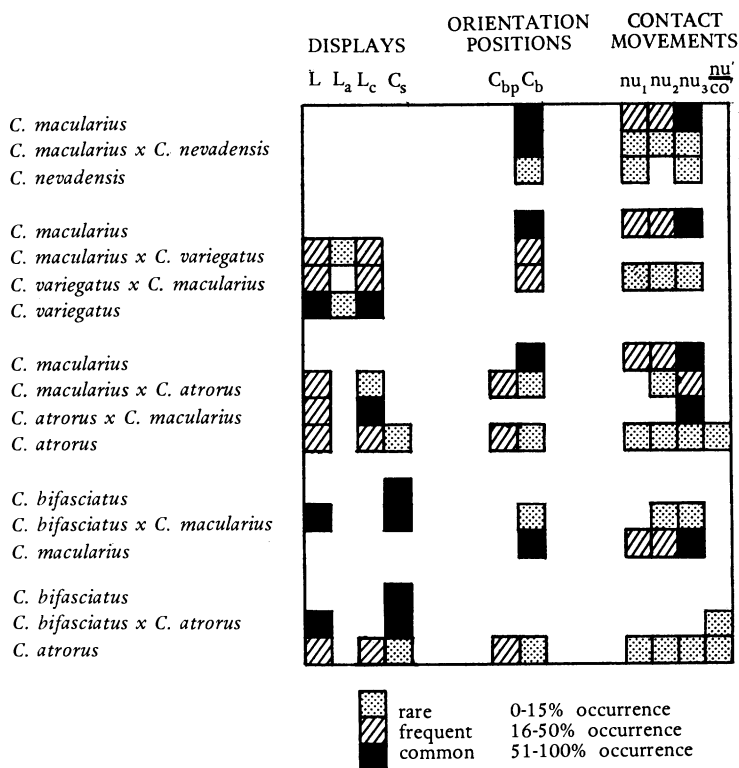
*macularius* and *C. atrorus*, but the number of observations in these cases was too small to preclude the possibility of scoring artifact. If present at all, parental motor patterns in hybrids were without apparent modification; with one possible exception, there were no discernably distinctive hybrid motor patterns. The possible exception is the presence of motor pattern "L" in *C. bifasciatus*  $\times$  *C. macularius* hybrids; this component was not detected in either parental species.

The inheritance of courtship behavior in *Cyprinodon* hybrids appears similar to that of

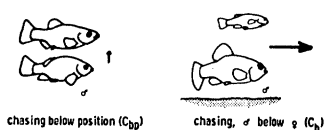
sexual behavior in the *Xiphophorus* hybrids studied by Franck (1970). Franck concluded that several premating motor patterns in *Xiphophorus* were under polygenic control; we have no data that contraindicate this hypothesis in *Cyprinodon*.

**Sex ratios.**—Aberrant sex ratios or unisexual sterility were noted in only three specific combinations of the 14 that were tested. These were:

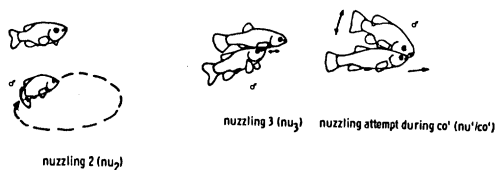
- 1) *C. atrorus*  $\times$  *C. macularius*: The first generation of this cross resulted in 20 females



## ORIENTATION POSITIONS



## CONTACT MOVEMENTS



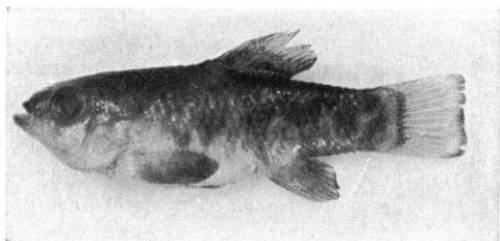


Fig. 5. "Masculinized" female  $F_1$  hybrid *C. macularius* (male)  $\times$  *C. variegatus*. This specimen was initially a functional female but gradually acquired the male secondary sex characteristics of darkened single fins, a black terminal caudal fin band and blue scales on the nape (visible in life only).

and one fertile male. The reciprocal cross produced a normal sex ratio.

- 2) *C. nevadensis*  $\times$  *C. variegatus*: All 32 hybrids were apparent females, but these were not tested for fertility. The reciprocal cross produced 12 sterile males and 24 females. Two of these females proved fertile when tested with males of *C. nevadensis*; the others were not tested.
- 3) *C. macularius*  $\times$  *C. variegatus*: Four females and one sterile male were produced. At least one of the females was fertile and was used in multispecific crosses (see below). An approximately reciprocal cross (*C. v. ovinus* as male parent) yielded a normal sex ratio. A functional female *C. macularius*  $\times$  *C. variegatus* hybrid gradually acquired a black caudal margin and blue iridescence on its nape (Fig. 5) which are the general male secondary sex characteristics of the genus. The gonads appeared grossly female upon dissection, but were not examined histologically. We also have noted occasional partial masculinization in older nonhybrid females of four *Cyprinodon* species.

The parental species in these three combinations are all members of different species complexes (as these are defined in Fig. 1). It might therefore be concluded that crosses between species complexes are more likely to yield aberrant

sex ratios or male sterility. In two combinations, however, these phenomena were found in only one of the pair of reciprocal crosses, and reciprocal crosses were attempted in only 4 out of 14 species combinations. Sterile progeny or aberrant sex ratios may be eventually discovered in some of the reciprocals of other crosses. More data are therefore required to support any relationship between parental divergence and the likelihood of sterility, aberrant sex ratios or similar phenomena in *Cyprinodon* hybrids.

A cross between *C. bifasciatus* and *C. nevadensis* yielded 5 females and no males, but the absence of one sex in a brood of five is not a statistically significant departure from a 1:1 sex ratio ( $\chi^2_{(1)} = 3.2, 0.1 > P > 0.05$ ).

The absent, rare, or sterile sex in *Cyprinodon* hybrids was invariably male. By application of "Haldane's rule" (Haldane, 1922) this observation suggests that the male is the heterogametic sex in at least some species of *Cyprinodon*, even though no cytological heterogamety has yet been detected in the genus.

Multispecific hybrids: Three multispecific crosses were attempted:

- 1) *C. rubrofluvialis*  $\times$  hybrid female *C. macularius*  $\times$  *C. variegatus*: This cross produced 20 progeny with a normal sex ratio (Fig. 6A); fertility was not evaluated.
- 2) Male hybrid *C. radiosus*  $\times$  *C. nevadensis*  $\times$  female hybrid *C. macularius*  $\times$  *C. variegatus*: This cross resulted in four females that strongly resembled *C. variegatus* (Fig. 6B). One of these females was testcrossed to a male *C. v. ovinus* and yielded more than 35 progeny of both sexes (Fig. 6C).
- 3) Male hybrid *C. macularius*  $\times$  *C. nevadensis*  $\times$  female hybrid *C. macularius*  $\times$  *C. variegatus*: Fewer than 5 young were raised from this cross; both sexes were present but their fertility was not evaluated.

These preliminary results suggest that multispecific pupfish hybrids are probably as easily produced and likely to be fertile as bispecific hybrids.

←

Fig. 4. Quantitative summary of male-specific components of courtship behavior in 6 *Cyprinodon* species and some of their  $F_1$  hybrids. The different displays, orientation positions, and contact movements are outlined in the lower portion of the figure. Note differences in courtship behavior of hybrid males from reciprocal crosses, especially hybrids of *C. macularius* and *C. atrovirens*; this contrasts strongly with the morphological similarity of these hybrids (see Fig. 3, "e" and "f"). Note also the presence of component "L" in *C. bifasciatus*  $\times$  *C. macularius* hybrids; although detected in several other species, this motor pattern was never seen in the courtship behavior of either parental species.



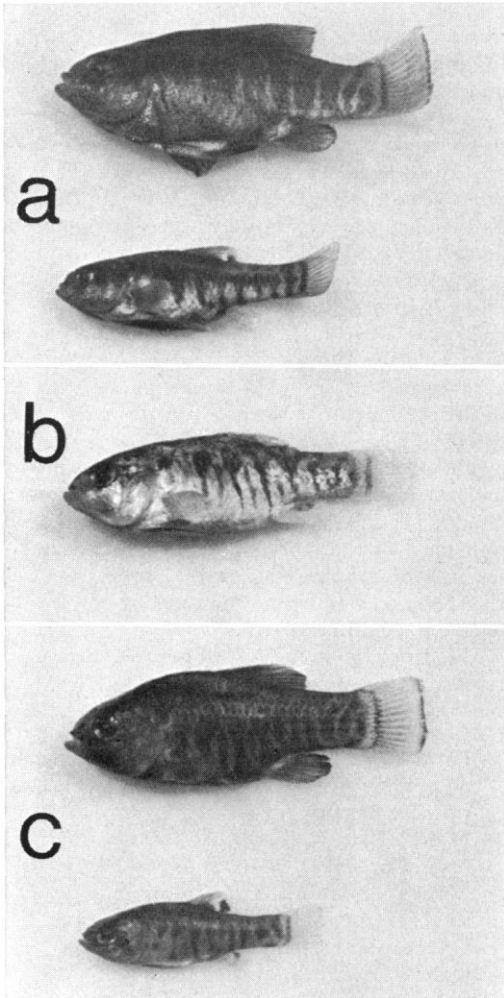


Fig. 6. Multispecific hybrids and their offspring. a) Triple hybrid: Male parent, *C. rubrofluvialis*; female parent:  $F_1$  hybrid (*C. macularius*  $\times$  *C. variegatus*). 34 mm S.L. male above, 29 mm S.L. female below. b) Fertile tetrahybrid females. 33 mm S.L. Male parents:  $F_1$  hybrid (*C. radiosus* (male)  $\times$  *C. nevadensis*). Female parent:  $F_1$  hybrid (*C. macularius* (male)  $\times$  *C. variegatus*). c) Progeny of female shown in "b" mated to a male *C. v. ovinus*; 35 mm S.L. male above, 24 mm S.L. female below.

Most of the hybrid specimens produced in this study are now preserved in the UCLA ichthyology collection (accession number W74-36).

#### DISCUSSION

We have obtained 18 bispecific hybrids from 14 different specific combinations within the genus *Cyprinodon*; the preponderant majority

of these laboratory hybrids were fertile. These data strongly suggest that adults could be reared from virtually any bispecific combination within the genus. The production of triple hybrids and a fertile tetra specific hybrid serves to emphasize further the remarkable interfertility of *Cyprinodon* species. Other cyprinodontids appear to be less interfertile than are *Cyprinodon* species. In the genus *Aphanius* and its allies some interspecific crosses yield fertile progeny of both sexes, but some combinations fail altogether or are associated with very high zygote mortality (Aksiray, 1952; Karbe, 1961; Öztan, 1954; Villwock, 1966). Well over half the large number of interspecific crosses thus far attempted in the African rivuline genera *Aphyosemion* (sensu lato) and *Epiplatys* fail during embryogenesis or yield progeny that do not attain sexual maturity (Scheel, 1968, 1972). A large number of interspecific crosses in the genus *Fundulus* have been attempted, and a significant number of these yield progeny that can be reared to adulthood (Drewry, 1967; Hubbs, 1970). However, a roughly equivalent number are successful in one direction only or fail during embryogenesis or soon after hatching. Thus, while interfertility of at least some species is well known in other cyprinodontid genera, the virtually total interfertility of *Cyprinodon* species is exceptional.

Specific interfertility seems to be more characteristic of poeciliids than of cyprinodontids, and interspecific hybridization has been strongly implicated in the ancestry of all gynogenetic poeciliid species (Abramoff et al., 1968; Schultz, 1973b). Among poeciliids the extraordinary interfertility of *Cyprinodon* species appears to be most closely approached by the species of the genus *Xiphophorus*. In that genus, bispecific hybrids between almost all of the 9–11 currently recognized species have been reported (Atz, 1962; Zander, 1962, 1968). Nearly all bispecific *Xiphophorus* hybrids are fertile, though reduced fertility is often encountered (Atz, 1962; Kallman, 1965). A few other poeciliid groups, e.g., certain species of *Poecilia*, (Schroeder, 1965) and *Poeciliopsis* (Schultz, 1966; Vrijenhoek and Schultz, 1974), appear to be interfertile to about the same extent as *Xiphophorus*.

Other teleost taxa that include a high proportion of interfertile species include *Lepomis* (Avisé and Smith, 1974), *Tilapia* (Fryer and Iles, 1972; Hickling, 1966), *Etheostoma* and its relatives (Hubbs, 1967), *Esox* (Buss and Miller, 1967), and many cyprinid genera (Duyvene-De Witt, 1964; Gilbert, 1964; Kobayasi, 1962).

However, none of these groups appears to display both the extreme interspecific genetic compatibility and the fertility of resultant hybrids that characterizes *Cyprinodon*.

Conventional wisdom in ichthyology assumes the existence of a strong correlation between the hybridization potential of species and their phylogenetic relationship (Nikolyukin, 1969; Suzuki, 1968; Hubbs, 1963, 1967, 1970; Hubbs and Drewry, 1959). This correlation is presumed to exist on almost any taxonomic level from above the ordinal to below the specific. Thus, in a given genus, species that can be hybridized are often regarded as more closely related than species that cannot be hybridized. Moreover, completely fertile interspecific hybrids supposedly indicate a closer relationship of their parental species than do partially fertile or sterile hybrids (Hubbs, 1967). The unusual interfertility of *Cyprinodon* species therefore appears to confirm the extremely close relationship among them that is suggested by electrophoretic (Turner, 1974; J. Darling, pers. commun.) and chromosomal (Stevenson, 1975; Miller and Echelle, 1975) data. However, there are at least some grounds for suggesting that *Cyprinodon* and other groups of species that hybridize readily (e.g., genera such as *Xiphophorus* and *Lepomis*) are not necessarily more closely related than otherwise roughly comparable groups where interspecific hybridization is not always successful (*Aphanius*, *Fundulus*, *Aphyosemion*). Two reasons for suggesting a more conservative approach to the relationship between genetic relatedness and hybridization potential in *Cyprinodon* follow:

- 1) *Lepomis* species are highly interfertile. However, a recent electrophoretic analysis of several *Lepomis* species by Avise and Smith (1974) showed that these species have very different gene pools and noted that "Close genic similarity is not a necessary corollary of hybridizing propensity."

- 2) So far as we are aware, *Cyprinodon* species are not significantly divergent in egg size or yolk content. Significant differences in the size or relative yolk content of the ova of presumptive parental species can lead to lethal anomalies in fertilization or subsequent development of hybrid zygotes (Hubbs and Drewry, 1959; Merriener, 1971a, 1971b; Schultz, 1973a). These anomalies are often detected by the failure in one direction of a reciprocal hybridization experiment; they are therefore not phylogenetic in nature. Hybridization failure would be more common, and species might falsely appear more

genetically distinctive, in groups where morphology or size of ova has been a significant evolutionary variable than in groups where this was not the case. The successful hybridization of *Cyprinodon* species may in part be a function of their similarity in egg morphology rather than an indication of exceptionally close genetic relationships.

Ultimately, the success of interspecific crosses in genera such as *Cyprinodon* may be related not to genetic or phylogenetic similarity, but to a lack of divergence in a relatively few genes encoding or controlling a small number of critical developmental processes or that are active during especially sensitive embryonic stages.

There are two implications of the virtually complete interspecific genetic compatibility of *Cyprinodon* species that may be of general importance:

- 1) Many completely interfertile species have been geographically isolated for relatively long periods—some, like *C. atrorus* and *C. macularius*, probably since the middle or late Tertiary (Liu, 1969). This immediately suggests that the evolution of postmating isolating mechanisms is not a necessary consequence of even prolonged geographical isolation and well-marked divergence in morphology and behavior; it may not occur at all in some groups.

- 2) In an analysis that compared the immunological distances of several proteins, the morphological divergences and the hybridization potential of anurans, birds and mammals, Prager and Wilson (1975) concluded that morphological divergence and loss of hybridization potential were both based primarily upon "regulatory evolution," i.e., changes in regulatory systems governing gene expression. *Cyprinodon* species have certainly evolved morphologically, yet have obviously not lost hybridization potential. This implies that morphological evolution and loss of hybridization potential are not necessarily coupled, and consequently that there may be more than one kind of regulatory evolution.

The systematic implications of the hybridization propensity of *Cyprinodon* species are difficult to evaluate. Obviously, our "no choice" or "forced" laboratory hybridization experiments do not critically assess the existence of traits that might function efficiently as premating isolating mechanisms if two interfertile species were to become sympatric. Interspecific hybrids have been collected in nature in two cases where sympatry of *Cyprinodon* species has re-

sulted from the actions of man, viz. *C. atrorus* and *C. bifasciatus* in Cuatro Ciénegas, Mexico (Miller, 1968) and *C. elegans* and *C. variegatus* in Lake Balmorhea, Texas (Stevenson and Buchanan, 1973). In both cases, however, there is some evidence which suggests that complete introgression is actively selected against or limited to recognizably "intermediate" habitats. There is an obvious need for more sophisticated laboratory and field studies to identify potential isolating mechanisms within the genus. For the moment, it seems apparent that, at least within the context of the genus *Cyprinodon*, the ability to hybridize presumptive species that are morphologically distinct is not critical evidence against their specific status.

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- LABORATORY OF FISHERIES AND MARINE BIOLOGY, DEPT. OF BIOLOGY, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90024. PRESENT ADDRESS (BJT): MUSEUM OF ZOOLOGY, UNIVERSITY OF MICHIGAN, ANN ARBOR, MICHIGAN 48109. Accepted 23 April 1976.

## Notes added in proof

1) While this paper was in press, four new species of *Cyprinodon* have been described (Miller, R. R., 1976. Four new pupfishes of the genus *Cyprinodon* from Mexico, with a key to the *C. eximius* complex. Bull. So. Calif. Acad. Sci., 75:68-75.) Species 17, 18, and 19 on our map (Fig. 1) are now *C. meeki*, *C. macrolepis* and *C. alvarezi*, respectively. The fourth new species, *C. nazas*, from the basins of the Rio Nazas, Rio Aguanaval and Laguna Santiagullo, was unknown to us and is not on the map. The extinct Devil's River pupfish (species 14 in Fig. 1) is now regarded as a differentiated population (subspecies?) of *C. eximius*.

2) We have learned that J. Cokendolpher, Midwestern State University, Wichita Falls, Texas, has also been investigating interspecific hybridization in *Cyprinodon* and has obtained  $F_1$  and some  $F_2$  and backcross hybrids from 8 different bispecific combinations of the following species: *C. atrorus*, *C. bovinus*, *C. macularius*, *C. nevadensis*, *C. rubrofluvialis* and an undescribed species from Chihuahua, Mexico. The vast majority of the hybrid progeny are fertile. His results therefore confirm and extend our own and we thank him for allowing us to mention them.

3) Kennedy (Copeia 1977:93-103) reports hybridization between *C. bovinus* and introduced *C. variegatus*.