Hybridization Experiments with the Genus Cyprinodon (Teleostei: Cyprinodontidae)

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HYBRIDIZATION EXPERIMENTS WITH THE GENUS *CYPRINODON* (TELEOSTEI: CYPRINODONTIDAE).—Multispecific hybridization of killifish has been reported primarily for *Fundulus* and Old World genera (Aksiray, 1952; Drewry, 1967; Öztan, 1954; Scheel, 1968; Villwock, 1958 and 1960; Karbe, 1961), little has been published on crosses in the genus *Cyprinodon* (Miller, 1948; Hubbs and Drewry, 1959; Stevenson and Buchanan, 1973; Cokendolpher, 1975; Turner and Liu, 1977; Kennedy, 1977) or on intergeneric crosses of New World cyprinodonts (Hubbs and Drewry, 1959, 1962; Hubbs, 1967). The present data confirm Turner and Liu (1977) in the remarkable extent in which the morphologically and behaviorally distinctive species of *Cyprinodon* are interfertile, while hybrids of other cyprinodonts have shown a high reduction in fertility, particularly in certain reciprocal crosses, and in many cases a high degree of F_1 ova mortality (Aksiray, 1952; Öztan, 1954; Karbe, 1961; Drewry, 1967; Scheel, 1968). The high degree of interfertility of *Cyprinodon* can in part be explained by the similar physiologic needs of the gametes, fertilized ova, larvae and adults, with essentially indistinguishable chromosome complements of species thus far tested also promoting a higher degree of interfertility (Turner and Liu, 1977; M. M. Stevenson, per. comm.)

Materials and methods.—Live material used for hybridization experiments was as follows (abbreviations used in Table 1 in parentheses): 1) *Cyprinodon atrorus* Miller (Atr), Cuatro Ciénegas Basin, Coahuila, México; 2) *Cyprinodon bovinus* Baird and Girard (Bov), Leon Creek, 18.9 km N. Fort Stockton, Pecos Co., Texas; 3) *Cyprinodon eximius* Girard (Exi), Alamito Creek, 7.2 km E.S.E. Presidio, Presidio Co., Texas; 4) *Cyprinodon macularius macularius* Baird and Girard (Mac-ma), Quitobaquito Springs, Organ Pipe Cactus National Monument, Pima Co., Arizona; 5) *Cyprinodon macularius californicus* Girard (Mac-ca), Whitefield Creek, N.E. shore Salton Sea, Riverside Co., California; 6) *Cyprinodon nevadensis amargosae* Miller (Nev-am), Amargosa River, S.E. corner of Death Valley National Monument, San Bernardino Co., California; 7) *Cyprinodon nevadensis nevadensis* Eigenmann and Eigenmann (Nev-nv), Saratoga Springs, Death

TABLE 1. RESULTS OF INTRAGENERIC *Cyprinodon* HYBRIDIZATION.

Cross*	Immatures	Males	Females	Comments
Mac-caAtr	30	3	6	
(Mac-caAtr)F2	2	2		
(Mac-caAtr)Atr	3	1	2	♂ mortality due to deformity of head
SalAtr	37			Pre-adult mortality due to "dropsy"
Nev-nvAtr	11	3	7	Adult mortality due to "dropsy"
AtrNev-am	17	1	2	♂ has no pelvic fins
RubAtr	32		25	Hybird very large and robust
(RubAtr)Rub	25	1	1	
((RubAtr)Rub)F2	1	1		
(RubAtr)Atr	24	3	9	High larval mortality due to deformed spines
((RubAtr)Atr)F2	24			No development beyond larval stage- never feed
((RubAtr)Atr)Rub	37			No development beyond larval stage- never feed
((RubAtr)Rub)((RubAtr)Atr)	15			No development beyond larval stage- never feed
(RubAtr)((RubAtr)Atr)	37			No development beyond larval stage- never feed
(RubAtr)Nev-am	30	2	1	
(RubAtr)(Nev-nvRub)	1		1	
Mac-caVar	3			No development beyond larval stage- never feed
VarPec	2		2	
VarExi	22	4	9	
Mac-caExi	7		6	
RubExi	13		13	No resistance to temperatures below 15 C
RubSp	35		13	
RubPec	30		4	Weak, slow growth with high losses
PecRub	31	7	12	
(PecRub)F2	54	1	3	Immature mortality due to "dropsy," mortality of one ♀ due to deformities of head and spine, 2 intersexes
Rub(PecRub)	21		1	Growth slow with high losses
BovRub	9	3	6	
Bov(BovRub)	1	1		Head deformed
RubNev-am	50	1	2	One ♀ without ventral scales on abdomen
RubNev-nv	47	1	19	Mortality due to "dropsy"
Nev-nvRub	12	1	7	♀ and immature mortality due to "dropsy"
(RubNev-nv)Rub	2	1		
Rub(Nev-nvRub)	7	2	3	
Mac-caRub	7	5		
PecMac-ca	12		2	
PecMac-ma	7		2	Weak, mortality due to deformity of spine
Mac-caSp	50			No development beyond larval stage, seldom feed
BovSp	8		2	
(BovSp)Sp	3		1	

* See materials and methods for abbreviations used to denote crosses; maternal parental species listed first.

Valley National Monument, San Bernardino Co., California; 8) *Cyprinodon salinus* Miller (Sal), Salt Creek, Death Valley National Monument, Inyo Co., California; 9) *Cyprinodon rubrofluviatilis* Fowler (Rub), Gilbert Creek, 4.8 km S. Burkburnett, Wichita Co., Texas; Red River, 9.6 km S.E. Randlett, Cotton Co., Oklahoma (only hybrids with *C. eximius* and *C. pecosensis*); 10) *Cyprinodon variegatus variegatus* Lacépède (Var), Galveston Bay, Chambers Co., Texas (hybrids with *C. rubrofluviatilis* and *C. bovinus*); Lake Balmorhea, Reeves Co., Texas (hybrids with *C. eximius, C. macularius* and *C. pecosensis*); 11) *Cyprinodon* sp. (Sp), Ascención, Chihuahua, México; 12) *Cyprinodon pecosensis* Echelle and Echelle (Pec), Screw Bean Creek, 1.6 km W.S.W. Red Bluff Lake, Reeves Co., Texas; Mirror Lake, Bottomless Lakes State Park,

Chaves Co., New Mexico (only hybrids with *C. macularius*); 13) *Jordanella floridae* Goode and Bean (Flo), no data—aquarium fish. Voucher specimens of each stock are deposited in the Vertebrate Collection, Midwestern State University, Wichita Falls, Texas.

Hybrids were reared from fertilized ova collected from matings in aquaria, with heterospecific pairs used for primary matings. If available, up to four hybrid females were mated to single males. For aggressive pairs and group spawnings a 40 liter aquarium was employed, for non-aggressive pairs a 20 liter aquarium was used. All crosses were carried out in 10% synthetic marine water, except matings with *C. variegatus* (Galveston Bay stock) were in 50% synthetic marine water.

To promote spawning all specimens were under constant illumination. Temperatures in the aquaria were not controlled and fluctuated with the season and time of day (daytime temperatures ranged from 10 C in winter to 32 C in the summer). Each aquarium was furnished with "synthetic spawning mops" (Terceira, 1974). Fertilized ova were removed daily from the spawning media and placed in petri dishes for observation. Upon hatching, the larval fish were transferred to larger aquaria. Crosses resulting in pre-larval death were repeated with different specimens, while successful crosses were generally not replicated. As each parental species had been successfully bred in captivity over several generations no control was run.

The "dropsy" condition causing mortalities (Table 1) is regarded (Amlacher, 1970) as bacterial hemorrhagic septicemia. Following Goldstein (1971), the infected fish were treated with tetracycline as soon as noticeable extension of the scales was evident. Treatment was never successful, and it appears that the bacterial invasion is secondary to a hereditary deformity (Amlacher, 1970). All post-larval hybrids produced in this study are now deposited with the Hybrid Register of the American Killish Association (% Hybrid Registrar, Mr. Fran Clifford, 194 Grafton Avenue, Blasdell, New York 14219).

Results and discussion.—From April 1973 to November 1976, 57 crosses were attempted in aquaria between 15 populations of ten species of *Cyprinodon* and *Jordanella floridae*. Intergeneric bispecific crosses accounted for five (one reciprocal cross) of the attempted combinations. The intergeneric crosses FloRub (80 ova, 27 fry), FloPec (279 ova, 12 fry), Mac-caFlo (54 ova, 2 fry), and Nev-amFlo (47 ova, 46 fry) resulted in the production of fry which were weak and never feed. The cross PecFlo (37 and 50 ova) failed to produce ova that develop. One trispecific cross attempted between three species complexes resulted in production of embryos that did not hatch. The cross Mac-ca (AtrRub) was replicated on three different occasions with similar results of embryo mortality.

Of the 57 attempted combinations, 51 resulted in the production of hybrid larvae and fish. Of these, 46 were bispecific, four trispecific and one tetraspecific. Six of the bispecific crosses were carried to F_2. Juveniles with normal growth were produced in the following test crosses: (Nev-am(AtrRub))Rub, (Nev-amAtr)(SpBov), (Nev-amAtr)Atr, Nev-am(Nev-amRub), (Nev-amRub)Rub, ((RubNev-nv)Rub)F_2, (RubNev-nv)Mac-ca, (RubNev-nv)F_2, Rub(RubBov), (RubBov)Rub, VarRub, VarBov.

The results of the 39 remaining crosses are presented in Table 1. Although the majority of hybrids were fertile, there were a few combinations which resulted in partial sterility, intersexes, or the absence of one sex among the offspring. Abnormalities resulting in mortality were also noted in many combinations.

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HYBRIDIZATION IN THE GENUS *PSEUDOLABRUS* (LABRIDAE).—Hybridization between freshwater fish species is a widespread and relatively frequent occurrence. Several hundred such cases have been reported, many of them intergeneric hybrids. This field has been comprehensively reviewed by Hubbs (1955), and the hybrids listed by Slastenenko (1957) and again by Schwartz (1972). In contrast to this situation approximately 30 cases of hybridization between marine fishes have been reported, the majority of these between species of flatfishes or between some pelagic fishes. Reports of hybridization between marine reef species are even rarer, and the only well-documented cases appear to be between two acanthurids, *Acanthurus achilles* × *A. glaucopareius* (Randall, 1956), between two pomacanthids, *Holocanthus isabelita* × *H. ciliaris* (Feddern, 1968), and between a number of pairs of species in the genus *Chaetodon* (Burgess, 1974; Takeshita, 1976; Randall et al., 1977).

During the course of subtidal research work in north-eastern New Zealand, two fishes that appeared to be hybrids between species of the genus *Pseudolabrus* were observed and collected. A number of other suspected hybrids were also observed. In view of the apparent infrequency of marine fish hybridization it was thought worthwhile to report these cases in some detail. All the examples reported were found in the vicinity of Goat Island Bay, Leigh, where the University of Auckland maintains a marine laboratory. Meristic and morphological details of the two specimens collected were compared with ten individuals of a range of size of the probable parent species: *Pseudolabrus celidotus* and *P. fucicola*. The gonads of the hybrids were removed and sectioned to determine their reproductive capability.

Morphologically the species of *Pseudolabrus* are very similar and apart from coloration *P. celidotus* and *P. fucicola* differ in only four main features: number of pectoral rays, number of cheek scale rows, caudal fin shape and median fin length. A comparison of these features for

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Fig. 1. Photographs of collected specimens of the two parent species and the hybrid showing differences in fin shape and size. All specimens are terminal color phase fish (males): A) *Pseudolabrus celidotus*, B) *P. celidotus* × *P. fucicola* hybrid, C) *P. fucicola*.