RECIPROCAL HYBRIDIZATION BETWEEN THE CALIFORNIA AND GULF OF CALIFORNIA GRUNIONS, *LEURESTHES TENUIS* AND *LEURESTHES SARDINA* (ATHERINIDAE)

The California grunion, *Leuresthes tenuis*, and the Gulf of California grunion, *L. sardina,* are the only fishes that temporarily leave the water during spring high tides to deposit their eggs in beach sand (Walker 1952). The eggs develop in the nearly dry sand and hatch when uncovered and agitated by the surf of the next series of high tides.

The grunions have an allopatric distribution. The California grunion ranges from Monterey Bay, Calif., to Bahía Magdalena, Baja California Sur. The Gulf grunion is endemic to the Gulf of California, ranging from Bahía Concepción, Baja California Sur, and Guaymas, Sonora, Mexico to the mouth of the Río Colorado (Moffatt and Thomson 1975).

Recent comparisons show that morphological, physiological, and behavioral differences exist between the grunions. Morphologically very similar, the most diagnostic characterictics distinguishing them are lateral scale row counts; the mean number in *L. tenuis* is 75 and in *L. sardina* is 55. Gulf grunion adults are also significantly longer, more slender, have a smaller eye diameter, and are more lightly pigmented than those of the California grunion (Moffatt 1974; Moffatt and Thomson 1975). Gulf of California grunion have wider embryonic and larval thermal tolerances, a higher larval preferred temperature, and wider larval salinity tolerances (Reynolds and Thomson 1974a, b, c; Reynolds et al. 1976, 1977; Moffatt 1977).

Light response remains positive in Gulf grunion through adulthood, whereas the response shifts from positive in the larvae to negative in the adults of the California grunion (Walker 1952; Reynolds and Thomson 1974c; Reynolds et al. 1977). In response to the shorter wave period in the northern Gulf of California, the duration of the spawning act of the Gulf grunion females is much briefer than that of the California grunion females (Thomson and Muench 1976; Muench 1977).

Only recently has the congeneric status of the grunions been recognized (Moffatt 1974; Moffatt and Thomson 1975). Evidence to date indicates that the California grunion, the less primitive of the two species, has adapted to the less fluctuating tidal and thermal regimes of the California coast, following isolation from an ancestral type by the

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Baja California peninsula (Moffatt and Thomson 1975; Moffatt 1977).

Hybridization and hybrid survival experiments have been widely used as indices of divergence and have made valuable contributions as a tool in the definition of phylogenetic relationships (Hubbs 1967, 1970). In an attempt to further illuminate the relationship between the grunions, we made artificial and reciprocal crosses and we report on the first successful reciprocal hybridization of *Leuresthes tenuis* and *L. sardina*.

Materials and Methods

Adult grunions, although easily obtained in large numbers, are difficult to maintain and transport alive. On 18 March 1976 (2330 PST), milt from six California grunion males was collected at Scripps Beach, La Jolla Calif., mixed in the beaten yolks of two hen eggs (Bratanov and Dikov 1961), and transported to El Golfo de Santa Clara, Sonora, Mexico. The milt-yolk mixture, maintained between 16° and 20°C, was used to fertilize the eggs from 8 to 10 Gulf grunion females obtained at El Golfo on the following day (19 March) during a spawning run which began about 1700 MST. During this same run Gulf grunion milt was collected from 6 to 7 males, transported in the same manner and used to fertilize California grunion eggs from about 10 females obtained during a run that night at La Jolla at 0115 PST (20 March). One prior and four subsequent attempts to hybridize the grunions were made during the 1975, 1976, and 1977 spawning seasons, but these were unsuccessful because one or both grunions failed to spawn.

The female grunions were rinsed thoroughly in clean seawater before their eggs were stripped directly into the milt-yolk mixture. The mixture was diluted slightly with fresh seawater to increase sperm motility, gently agitated, and kept cool until the end of the spawning run. The eggs were then strained, rinsed with seawater, and placed in plastic refrigerator containers between moist paper towels for transport and incubation.

Conspecific control embryos of each species were obtained by mixing eggs and milt in a bucket of seawater, one-third full, and did not involve the transportation or preservation of milt in hen yolk. When spawning individuals were plentiful, as at the Gulf grunion run on 19 March, six to nine males were stripped per one female in order to achieve maximum fertilization levels (Moffatt 1977).

Both sets of hybrid fertilized eggs and the conspecific controls of *L. tenuis* (18 March) and *L. sardina* (19 March) were transported from San Diego, Calif., to the University of Arizona at Tucson aboard commercial airlines. Upon arrival (22 h postfertilization in *L. sardina* × *L. tenuis* and *L. sardina* controls; 13 h in *L. tenuis* × *L. sardina;* and 32 h in *L. tenuis* controls) each set of eggs was inspected. Their development was monitored daily thereafter.

Both California grunion spawning runs were sparse at La Jolla. Therefore, the greatest portion of eggs and sperm available were devoted to the hybridization experiments and a low conspecific *L. tenuis* sample size resulted. Consequently, the developmental and hatching data reported herein for these embryos are a compilation of these few controls and egg sets obtained on other occasions, incubated at 20°C from 12 h postfertilization (Moffatt 1977).

Yolk-sac larvae of the two hybrids and the conspecific controls were placed in separate tanks containing artificial seawater and raised on newly hatched *Artemia*, freeze-dried marine zooplankton, commercial staple food, and frozen *Artemia* nauplii. Larvae of the hybrids and controls were maintained for nearly 5 mo although initial mortality rates (first 2 mo) in all groups were high (>90%). On 19 August, 141 days posthatching, the aquaria air lines were fouled by compressor oil and the few remaining hybrids and controls died. Only two *L. tenuis* × *L. sardina* and nine *L. sardina* × *L. tenuis* individuals survived to a size (>12 mm) at which the scale rows could be counted. This is not to imply that scales might not have been present prior to this time, merely that no attempt was made to count them.

Results

At 22 h postfertilization, cleavage had progressed to the gastrula stage in *L. sardina* × *L. tenuis* embryos as it had in the *L. sardina* controls. The *L. tenuis* × *L. sardina* hybrids had reached a 32-cell blastodic stage at 13 h postfertilization as do *L. tenuis* embryos.

Artificial fertilization levels in the conspecific controls fell between 85 and 99% during the peaks of their spawning seasons when male to female ratios of 6 or 9:1 were available. The fertilization

levels of both hybrids ranged from 60 to 70%. These diminished levels in the hybrids may have resulted from a combination of several factors such as: the low male to female ratios used (<1:1); decreased sperm motility in the viscous hen-yolk medium; high sperm mortality due to time, starvation, temperature shock, handling, etc. or partial reproductive isolation between the species in the form of mild fertilization block to nonconspecific spermatozoa.

The grunions, *L. tenuis* and *L. sardina*, showed similar developmental rates (Moffatt 1977). Development proceeded normally in the hybrids and at about the same rate as the controls. No unusual embryonic mortality was observed in the hybrids, evidence that these embryos were not gynogenetic hybrids (Moore 19.¯.).

Preliminary trials showed that hen's yolk and seawater alone will not initiate cleavage in Gulf grunion eggs. Precautions were taken to prevent conspecific milt contamination. Preliminary examination of cellular nuclei smears of developing embryos immersed in colchichine revealed somatic chromosome numbers of about $2n = 40$ in all four sets of embryos (controls and hybrids), further evidence that these embryos were true diploid hybrids.

Grunion embryos will hatch after vigorous agitation in seawater. On 31 March at 284 h (11.8 days) postfertilization, 65.6% of the *L. sardina* × *L. tenuis* embryos hatched and 66.5% of the *L. tenuis* × *L. sardina* embryos hatched at 272 h (11.4 days). These hatch times are similar to those of the controls. Hatching can be induced in both grunions at 10.2 days postfertilization when embryos are incubated at 20°C (Moffatt 1977).

Newly hatched *L. tenuis* larvae are typically more darkly pigmented; they have a larger eye diameter; they are stronger swimmers; and they are more capable of escaping net capture than newly hatched *L. sardina* larvae (Moffatt 1977). *Leuresthes tenuis* larvae are 10% longer (mean total length = 7.70 mm) than those of *L. sardina* (mean total length = 6.93 mm). The greater length of the California grunion yolk-sac larvae occurs in the postanal region as in the adults. California grunion larvae are also 52% heavier (mean dry weight = 0.340 mg) whereas, the mean dry weight of *L. sardina* equals 0.223 mg (Moffatt 1977). The greater length and weight of the California grunion at hatching may be attributable to the 4.10 times greater ovum volume (Moffatt 1977; Moffatt and Thomson in press).

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These differences which distinguish the prolarvae of *L. tenuis* and *L. sardina* were also observed in the hybrids. In most characteristics the *L. tenuis* × *L. sardina* larvae were not visibly distinguishable from the maternal controls (*L. tenuis*), e.g., size, pigmentation, and swimming ability. However, the *L. sardina* × *L. tenuis* larvae appeared to be somewhat intermediate to the controls in extent of pigmentation and swimming ability. At 2 wk after hatching the length and pigment differences between the larvae were more pronounced. Premaxillary teeth were visible in the *L. sardina* × *L. tenuis* larvae but not in the reciprocal hybrids. Again, hybrids closely resembled the maternal controls. Gulf grunion adults typically have much stronger dentition than do the adults of the California grunion (Moffatt and Thomson 1975).

As previously mentioned, the most diagnostic differences between the adult grunions are the lateral scale row counts. Scale counts of the 141-day-old controls were essentially the same as those of the adults (Table 1). The counts of the hybrids were intermediate and significantly different from each other. Those shown by both hybrids were significantly different from those of both parental species. The lateral scale rows of the hybrids were closer in number to those of the maternal controls. Mean counts of *L. sardina* × *L. tenuis* were 32% closer to those of *L. sardina*; and *L. tenuis* × *L. sardina* were 20% closer to *L. tenuis* than to those of the paternal parents, *L. tenuis* and *L. sardina*, respectively.[1] The intermediate counts indicate paternal genome influence and that these are indeed diploid hybrids.

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[1] A mean hybrid count greater or less than 65 (the midvalue between the parental species) indicated the affinity to one parent or the other. The numerical affinities (percentages) were calculated as the ratio of the differences between 65 and the hybrid count and between 65 and the adult counts.

TABLE 1.—Means, ranges, *n*, and *P* values of lateral scale row counts observed in 141-day-old hybrids and controls and adults of the grunions, *Leuresthes tenuis* and *L. sardina*.

Parents	*L. tenuis*			*L. sardina*		
	Juveniles	Adults	P	Juveniles	Adults	P
L. tenuis	x 75.5	x 74.6		x 61.8*		
	(75-76)	(69-80)	0.6	(61-63)	—	—
	n 2	n 143		n 9		
L. sardina	x 67.0*			x 55.1	x 55.3	
	(66-68)	—	—	(54-57)	(51-60)	0.7
	n 2			n 9	n 177	
	P 0.01	—		P 0.001	—	

*Student's *t*-test comparison of the lateral scale row counts between the two hybrids P 0.001.

Discussion

Natural hybridizations are reportedly more common among freshwater fishes than among marine fishes (Hubbs 1955). Hubbs (1970) stated that, "teleost hybrids are relatively easily produced and if the parental morphology is similar the hybrids are easily reared." The results of natural and artificial amphibian and teleost crosses have been widely employed for estimating degrees of phylogenetic divergence, revealing systematic patterns, and explaining mechanisms controlling development and differentiation.

Davidson (1968) reports that the closer the phylogenetic relationship between the species hybridized, the less likely the hybrid genome control will be displayed early in development. This is because the mechanical aspects of early development tend to be similar in closely related species and may be primarily under the control of maternal RNA accumulated in the egg prior to fertilization. Davidson believes this, at least in part, accounts for the commonly observed resemblance of hybrids in early developmental stages to the maternal parent. The genetic influence of the paternal genes in the hybrid genome may not be apparent phenotypically until long after the onset of differentiation (Davidson 1968).

It is possible that such mechanisms account for the maternal resemblance pattern observed in the grunion hybrids as well. The hybrids resembled the maternal parents in overall size and body proportions, coloration, swimming ability, net-escape capability, and dentition until long after hatching. Only when the lateral scale rows were counted at 141 days after hatching did the influence of paternal genes become visibly and quantitatively apparent.

The numerous artificial and two natural hybridizations (interspecific and intergeneric) reported among the Atherinidae are reviewed by Hubbs and Drewry (1959), Rubinoff (1961), and Hubbs (1970). Natural hybrids reported between *Menidia menidia* and *M. beryllina* along the Atlantic coast of Florida (Gosline 1948) exhibit intermediate counts (i.e., scales and fin rays). Most of the experimental crosses between these atherinids resulted in low developmental success and low survival rates except those of *M. beryllina* ♀ × *M. menidia* ♂ (Rubinoff 1961). Rubinoff did not report whether any intermediate characteristics existed in these hybrids nor was the reciprocal cross attempted.

Geographically isolated species forms adapt to their respective environments by the evolution of appropriate gene complexes. Then, if sympatry reccurs and hybridization takes place, hybrid individuals will usually be selected against (Mayr 1963; Ford 1964). Hybrids not selected against will usually be successful over only a narrow geographical range, since in animals, natural hybridization is commonly associated with environmental perturbation (Mayr 1963; Manwell and Baker 1970).

The *Menidia* species are sympatric and hybridization does occur in northern Florida, a very narrow portion of the overlap in their ranges (Gosline 1948). Like these species, grunions are marine fishes with similar, but not identical, ecological preferences. However, the grunions are allopatric and natural hybridization is not possible.

According to Mayr (1963), some investigators argue that renewed sympatry with hybridization is required as a process of speciation in order to "perfect isolating mechanisms," and, therefore, unlike the *Menidia* species, the heterospecific status of the grunions may be questioned, especially in light of the hybridization success reported herein. We conclude that, despite our success at hybridizing *L. tenuis* and *L. sardina*, the morphological, physiological, and behavioral distinctions between them warrant their continued recognition as separate species.

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TYCHOPLANKTONIC BLOODWORM, *GLYCERA DIBRANCHIATA*, IN SULLIVAN HARBOR, MAINE

The bloodworm, *Glycera dibranchiata*, is distributed from the Gulf of St. Lawrence to the Gulf of Mexico and from central California to lower California and Mexico. It occurs from intertidal water to 402 m depth (Pettibone 1963), but it is more abundant in shallow coastal water. In Maine and Nova Scotia the worms are dug commercially along the coast from the upper layers of the intertidal sand-silt-clay strata (Dow and Creaser 1970; Anonymous 1974; Glidden[1]).

Spawning bloodworms are briefly pelagic occurring in large numbers as they swarm in the afternoon. Creaser (1973) observed swarming in Maine during June. Simpson (1962) reported swarming both in June and November-December, suggesting a biannual spawning in Maryland. Klawe and Dickie (1957) did not observe swarming by bloodworms in Nova Scotia, although other evidence indicated that the worms spawned in mid-May. They suggested that the worms had a short nocturnal swarming period making them difficult to observe. Simpson (1962) checked this possibility

[1]Glidden, P. E. 1951. Three commercially important polychaete marine worms from Maine: *Nereis (Neanthes) virens, Glycera dibranchiata, Glycera americana.* Rep. to Maine Dep. Sea Shore Fish., Augusta, Maine.