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TELEOST HYBRIDIZATION STUDIES

By

Clark Hubbs

The University of Texas at Austin 78712

"Traditionally, studies such as ours have been based on morphology, especially the skeleton, which is the only complete organ system available for detailed comparisons with fossils. However, with the variety of both primitive and advanced teleosts living today, we are most emphatically of the opinion that approaches other than morphological ones would be exceedingly fruitful in the investigation of teleostean interrelationships."

The above quotation from Greenwood *et al.* (1966) clearly states George S. Myers' philosophy that systematic studies are central to biology. Any difference or similarity between two groups of organisms can be of value in estimating the amount of divergence; therefore, all biologic investigations can provide direct or indirect taxonomic information. Similar or identical organisms should be used in order to obtain repeatable experimental results; therefore, all biological investigations can be considered to be based on systematic research.

At the present time, the classification of most major taxa is based on their gross anatomy. Although this is due primarily to tradition, there is a valid scientific basis. Most experimental* analyses cover such small fractions of the taxonomic subdivisions that experiment-based classifications would have major gaps. Moreover, a typological concept has no place in modern systematics.

* Hereafter the word experimental should be considered to equal all types of analyses that are not traditional studies of museum specimens.

When we contrast several related taxa we should compare the *spectra of attributes* in the diverse members of each taxon. Because of the difficulty of obtaining data and the short history of such studies, most experimental investigations contrast supposed typical representative members of the taxa to be compared (= typology).

In contrast, experimental studies are not burdened with traditional taxonomic relationships. The refreshing new viewpoint can challenge the validity of an unsupported traditional taxonomic conclusion. The resulting interaction between morphological taxonomists and experimentalists can provide a realistic arrangement of organisms approximating their phylogenetic relationships. The primary contribution of the taxonomist to this interaction may be to point out taxonomic problem groups so that the experimental biologist can concentrate his efforts efficiently.

Among the host of problems faced by the ichthyologist concerned with taxonomic problems has been the separation of environmental and genetic factors. Tåning (1952) and many others have shown that a single environmental variable can concurrently alter several morphologic entities regardless of genotype. Therefore, a morphologic comparison that emphasizes those attributes might produce a dichotomy between those fishes reared in warm water and those reared in cold water, despite their genetic affinities.

Environment-related problems can further plague taxonomy because survival in similar environments tends to select for similar morphologic attributes. For example, fishes that live in rock crevices tend to be elongate. Existence in this environment seems also to be enhanced by small eyes and scales. Some may be blind or naked. Many also have anteriorly located or reduced pelvic fins and small gill slits. Theoretically, distortions of true relationships by convergent or parallel evolution can be resolved by the use of diverse attributes. Practically, one must be careful not to use apparently divergent characters that happen to have selective value in similar habitats. Use of "non-adaptive" characters for taxonomy should help resolve problems of this type, but can one be certain that any character is not adaptive?

Johnson and Wicks (1964) have advocated the use of molecular biologic (= electrophoretic) studies because they may provide the "ultimate" information on relationships. Studies of DNA hybridization seem to offer even more promise of approximating the degree of phylogenetic divergence. Even this "ultimate" systematic tool may have potential weakness. Assume that we have two ancestral species of omnivorous fishes occupying estuaries. Both evolve into a freshwater herbivore and a saltwater carnivore. The DNA sequences in each species pair would diverge so that each saltwater type would have a sequence of codings favorable for survival in high salinity. Similarly each would have their DNA controlling their digestive enzymes designed to break down animal ma-

terial. The freshwater representatives of each pair would have their nucleotide sequences designed to produce enzymes different from their sibling species, but the same as those of their more distantly related ecological counterpart. The above simplified model is undoubtedly extreme, but may indicate how dependence on a single analysis could be hazardous. It is also possible that this type of convergence would be missed despite the type of analysis used.

In effect, we have returned to the premise that taxonomic conclusions should be based on the sum (or product?) of the biological studies available. Nevertheless, each investigator should not attempt to carry out investigations in all areas, but should concentrate on those for which his aptitude, experience, and interest suit him. During recent years, I have used hybrid survival as an index of phylogenetic relationship. This approach has three major merits: 1) It necessitates minimal expenditures, 2) It measures genetic divergence, and 3) The results approximate those of classical morphological taxonomy.

The general agreement between hybrid survival experiments and classical fish taxonomy (Hubbs, 1967) and the potential hazards in such tests (Hubbs and Drewry, 1960) combine to make such tests valuable contributions to, but not the ultimate answer for, problems of phylogenetic relationships. The experiments reported below relate to two levels of relationships: 1) Arrangement of fishes within the family Cyprinodontidae and 2) Arrangement of various fish families.

MATERIALS AND METHODS

The techniques of Strawn and Hubbs (1956) were used for removal and mixing of the gametes. Two modifications were used that reduced some of the experimental difficulties. Most of our experiments have used gametes from "wild" fish, that is, the individuals were removed from natural populations when nearly ripe and taken to the laboratory for the experiments. This necessitated hurried field work to avoid having eggs shed or becoming overripe (= stale) during transport. We have found that the gametes can be stripped and mixed in the field and then taken to the laboratory as they develop. Large numbers of experiments can be done in this manner if the trip is properly planned. We have used petri dishes for transportation of individual experiments. The ripe eggs attach to the surface of the basal unit and the top is held in place with rubber bands. A piece of tape on the edge of the basal unit permits water circulation, another on the bottom is used as a label. The sets of petri dishes are placed in styrofoam containers and the water changed when necessary. One still must be careful not to remain away from the laboratory for too long because careful examination of development is difficult in the field and newly hatched larvae can escape from the petri dishes. Keeping the transportation equipment cool prolongs the time that one can remain in the field.

We find that fertilization can be enhanced by use of mashed testes. Typically, semen is removed from males by coelomic pressure. Some species have such small quantities of semen that milt is seldom extruded by this technique, but eggs can be fertilized by extracting and mashing the testes. The same technique works with males whose sperm supply has been depleted in previous experiments.

When pertinent, the sources of the stocks will be presented with the listing of the experiments.

CROSSES OF CYPRINODONT FISHES

Moenkhaus (1910), Newman (1908), Hubbs and Drewry (1960 and 1962), Archer (1966), Drewry (1967), and others have reported on many successful crosses among species of *Fundulus* and with species of related genera such as *Adinia*, *Lucania*, *Rivulus*, *Jordanella*, *Crenichthys*, and *Cyprinodon*. Unfortunately, the last two listed papers are in thesis form and have limited circulation. In general, the level of success parallels the estimate of phylogenetic similarity as determined by morphological taxonomy. Most species of *Fundulus* can be crossed with the others and the hybrids reared to mature size. Two previously unlisted crosses, *F. seminolis* ♀ (Sumpter Lake, Florida) × *F. cingulatus* ♂ (Green Cove, Florida) and *F. seminolis* ♀ (Sumpter Lake, Florida) × *F. heteroclitus* ♂ (Matanzas Inlet, Florida) can be added to the extensive list of reared hybrids.

Many authors have reported that hybrids between *F. majalis* or its near relative (race?) *F. similis* and other species of *Fundulus* will hatch if *F. majalis* type sperm is used and not if *F. majalis* type eggs are used. Six tests with *F. zebrinus* (Iraan, Texas) sperm and *F. similis* (9 mile pond, Texas) eggs had over 100 fertilized eggs fail to hatch.

Drewry (1967) reported difficulties in crossing *F. notatus* or *F. olivaceus* with other species of *Fundulus*, but hybrids between them are easily reared (Thomerson, 1967). Drewry reported that only 1 of the 17 hybrids using *F. olivaceus* sperm (none available with *F. notatus*) hatched and it died shortly. The reciprocal experiments had 9 fertilized eggs of which 6 hatched but were not reared, indicating a difference between reciprocals that more recent results support. Archer (1966) also failed to rear hybrids between *Fundulus notatus* or *F. olivaceus* and 2 other *Fundulus* species. *Fundulus notatus* ♀ (Blanco R., Texas) has been crossed with *F. kansae* ♂ (Colbert, Oklahoma) and *F. grandis* ♂ (Port Aransas, Texas) and one fish from 8 and 5 fertilized eggs respectively reared, but both were deformed. *Fundulus olivaceus* ♀ (Scraper Park, Oklahoma) × *F. kansae* ♂ (Colbert, Oklahoma) had 13 of 18 eggs hatch but the deformed larvae shortly died. The difficulty of rearing the hybrids supports Drewry's observations, but they can be reared. The reciprocal hybrids are much more difficult

to rear. *Fundulus kansae* ♀ (Colbert, Oklahoma) × *F. olivaceus* ♂ (Scraper Park, Oklahoma) (twice); *F. kansae* ♀ (Colbert, Oklahoma) × *F. notatus* ♂ (Blanco R., Texas) (12 times); *F. kansae* ♀ (Miller Cr., Texas) × *F. notatus* ♂ (Little Piney Cr., Texas), *F. zebrinus* ♀ (Iraan, Texas) × *F. notatus* ♂ (Onion Cr., Texas) (5 times); *F. cingulatus* ♀ (Dog Lake, Florida) × *F. olivaceus* ♂ (Baker, Florida), and *F. similis* ♀ (Port Aransas, Texas) × *F. notatus* ♂ (Blanco River, Texas) all failed to develop late embryos. The "greatest level of success" achieved in these 22 tests was 1 egg (*F. kansae* Colbert × *F. notatus* Blanco) that produced chromatophores but did not show indication of gastrulation. The low success and high frequency of abnormalities supports Drewry's hypothesis that *F. notatus* and *F. olivaceus* are phylogenetically similar to each other but dissimilar to other species of *Fundulus*. Six of 7 attempts to cross *F. notatus* and *F. olivaceus* were successful but only 4 of 6 control tests.

The previous intrageneric hybridization tests have not used the West Coast species, *F. parvipinnis* (Mission Bay, California, population). Failure of crosses with *F. kansae* (both reciprocals), *F. grandis* sperm, and eggs of *F. cingulatus* (3 tests, 2 populations), *F. heteroclitus* (2 tests), *F. majalis*, *F. similis* and *F. olivaceus*, indicates that this species is separate from other species now placed in *Fundulus*. Of course, only 10 failures may not be enough tests to insure valid results. The better results of hybridizing *F. parvipinnis* with *Crenichthys baileyi* (Hubbs, 1967) may indicate a common ancestry.

Only Archer (1966) has previously reported hybridization tests with *Jordanella floridae*. He reported that one set of *Jordanella* eggs crossed with *Cyprinodon variegatus* sperm died as late embryos. Three tests with *Cyprinodon* females from Iraan, Texas, resulted in no fertilization, but 1 of 2 and 3 of 4 tests with female *Fundulus zebrinus* and *Lucania parva* respectively from the same locality produced late embryos, but none hatched. This indicates that *Jordanella* is distinct from those 3 species. Drewry (1967) reported difficulty in rearing hybrids between *Lucania* and *Fundulus*; however, Archer (1966) reared several *F. pulvereus* × *L. parva* individuals to adult size. Apparently the hybrids did not exhibit sexual dimorphism.

INTRA-FAMILIAL HYBRIDIZATION

The relationship of hybridization success to phylogenetic divergence of teleost families may be shown to have significant importance in the taxonomic arrangement of living fishes. In part this approach resurrects those of Moenkhaus (1910) and Hertwig (1936). The ability of producing late hybrid embryos in a series of cyprinodontid × atherinid crosses has been considered support of their close relationship (Rosen, 1964). One more combination can be added to the series already reported (Hubbs, 1967, and citations). Two of 15 eggs from *F. notatus* ♀ (Denison Dam, Texas) exposed to *Menidia audens* ♂ (U. Oklahoma

Biological Station) sperm gastrulated. Both produced heads, one was attached to the yolk mass and the other was free. Black pigments covered the yolk mass on both. The one with the head free also had extensive orange pigmentation and remained alive until almost all of the yolk was expended. These results resemble those of the reported series of cyprinodontid-atherinid hybrids. The failure at gastrulation of a parallel experiment with *Notemigonus crysoleucas* sperm shows that fundulines will not hybridize with all fishes.

Menidia audens has not previously been reported to have been tested in rearing experiments. The series (4) of controls done the same day all had 50 percent plus fertilization and hatching. Similar to most atherinids, the controls died a week later apparently due to starvation. Intrafamilial hybrids between *M. audens* ♀ and *Labidesthes sicculus* ♂ (Tishomingo, Oklahoma) (twice) had 25 percent–50 percent fertilization and all hatched and died with the maternal controls, showing that *Menidia* hybrids can be reared as far as the controls.

Menidia audens has also been tested for hybridization survival with members of several other families. The tests with *Notropis cornutus*, *Notemigonus crysoleucas* (3 times), *Gasterosteus aculeatus*, and *Aphredoderus sayanus* all terminated before gastrulation. Gastrulation and embryonic formation occurred in tests with a centrarchid and several percids. One egg gastrulated among 2 sets of *M. audens* eggs exposed to *Lepomis macrochirus* sperm. It died before pigmentation. Another set of *M. audens* eggs exposed to *Etheostoma radiosum* (Blue River, Oklahoma) sperm produced 5 early embryos, of which only 2 developed pigmentation, the test with *Percina caprodes* males from the same locality failed. Seven reciprocal experiments were set up with percid eggs from Blue River females, one *E. spectabile* and six *E. radiosum*. Six had some development and 58 of 192 gastrulated embryos produced pigmentation, but none showed any sign of circulatory development. Clearly these hybrids were more successful than most other interfamilial tests and approached that of the atherinid-cyprinodont tests.

A series of other intrafamilial tests were done with *Etheostoma* or *Hadropterus* eggs. It is not surprising that all 7 tests with the ostariophysines, *Moxostoma poecilurum*, *Notropis cornutus*, *Notropis umbratilis*, *Notemigonus crysoleucas*, and *Opsopoeodus emiliae* failed to gastrulate or that 3 crosses with the "black race" of *Gasterosteus aculeatus* (Chehalis, Washington) did not gastrulate. Five of 17 tests with *Aphredoderus sayanus* sperm had 1 or more eggs gastrulate. The males were from a stock obtained at Douglass, Texas. The successful combinations were essentially the same ones as the failures, indicating that the data are representative of the interfamilial combination. Two sets of *E. asprigene* (Douglass, Texas) eggs each had 2 eggs gastrulate and develop eye pigmentation. Two of them formed a heart that beat irregularly, but had no visible cells in the tubes. Many erythrocytes were present on the yolk mass anterior to the head. Another embryo had no heart beat but died breaking the egg shell.

Two sets of *H. scierus* (San Marcos and Pedro Creek, Texas) had 2 and 3 eggs gastrulate. Only 3 developed recognizable heads. Three additional embryos from an *E. spectabile* (San Marcos, Texas) $\times$ *A. sayanus* cross that developed pigmented eyes were sacrificed in an unsuccessful attempt to analyze the cytology. The putative hybrids were diploid but chromosome markers were not noted.

Four of 11 sets of *Etheostoma* eggs exposed to *Elassoma zonatum* sperm had gastrulation. All but one involved *E. spectabile* eggs. The 2 successful sets from Shoal Creek, Missouri, females had 6 gastrulated eggs, 5 had eye pigmentation, and 1 a functional heart beat and flow. All died at the time the maternal controls hatched. The 2 successful tests with Blue River, Oklahoma, females (*E. radiosum* and *E. spectabile*), each had 1 embryo that developed eye pigmentation.

The failure of 2 sets of *G. aculeatus* eggs exposed to *A. sayanus* sperm indicates that *A. sayanus* sperm will not fertilize all teleost eggs.

DISCUSSION

Most of the intrafamilial hybrid experiments substantiate those previously reported, i.e., teleost hybrids are relatively easily produced and if the parental morphology is similar the hybrids are easily reared. Drewry had evidence of a genetic block to the development of hybrids between *F. notatus* or *F. olivaceus* and other members of that genus. Because he had few tests (26 fertilized eggs), it might be possible that his results were due to chance. We had 26 fertilized eggs in the least studied reciprocal (*F. olivaceus* or *F. notatus* egg) and 22 tests with the reciprocal. Not only did the results confirm those of Drewry, they also showed a distinct difference between the reciprocals. Because most of our "standard *Fundulus*" in these tests were of the *F. kansae*—*F. zebrinus* type, it may be that the difference between reciprocals may relate only to those combinations. It is possible, however, that we have a second difference in reciprocal hybrid survival in funduline fishes. It is amply evident, however, that *F. notatus* and *F. olivaceus* are quite distinct from other species of *Fundulus*. Hybridization tests have clearly showed that gametes from *Adinia xenica* and *Lucania parva* are more compatible with gametes from typical *Fundulus* than are those of *Fundulus notatus* or *F. olivaceus*. Therefore, the genus should be expanded to include the members of *Lucania* and *Adinia*, or *F. notatus* and *F. olivaceus* should be separated from the other *Fundulus* species and placed in the genus *Zygonectes*.

The failure of 10 tests between *Fundulus parvipinnis* and 7 other species of *Fundulus* indicates that this species is quite distinct. The reasonable success of hybrids between *F. parvipinnis* and the *Crenichthys*-*Empetrichthys* complex suggests a possible relationship that makes biogeographic sense. The hybrids of all west coast Fundulines have earlier developmental blocks when crossed with an

east-coast representative than when crossed with another western type. Perhaps the Fundulines had an east-west primary separation. The western types then diverged into a coastal (*F. parvipinnis* precursor) and a desert spring (*Crenichthys*, *Empetrichthys*) type. Because *F. parvipinnis* occupied a "typical *Fundulus*" environment, it retained "typical *Fundulus*" morphology, while *Crenichthys* and *Empetrichthys* occupied clear warm spring waters and became superficially distinct.

Assuming that the cyprinodonts have lost spinous fin rays, it seems that hybrids between soft-rayed and spiny-rayed fishes die at or before gastrulation. The consistent failures indicate a uniform distant relationship and perhaps a treelike phylogeny rather than a bushy type suggested by Greenwood *et al.* A treelike phylogeny is also indicated by hybrid development to late embryonic stages in almost all crosses between two spiny rayed types. If we placed *Aphredoderus* and *Etheostoma* in separate superorders than the hybrid success is incongruous; in contrast, if we follow Regan and make *Aphredoderus* a relic of a group ancestral to other perciforms, the phylogeny would agree with the hybridization data.

The success of most interfamilial spiny rayed hybrids is quite similar. Only two types of combinations usually survive to hatching. The cyprinodont-atherinid hybrids and hybrids among percids, serranids, and centrarchids. Recent classifications place each unit in a suborder.

In the future, hybrid survival tests may aid in determining other family group relationships.

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This paper is dedicated to Dr. George S. Myers because of his inspired teaching of systematic ichthyology.

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