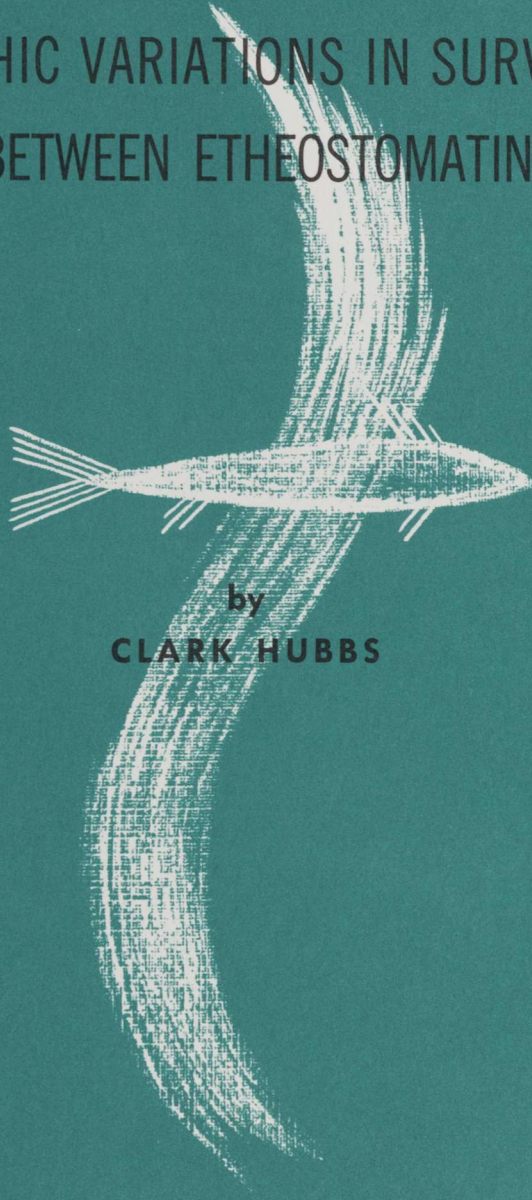


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**GEOGRAPHIC VARIATIONS IN SURVIVAL OF
HYBRIDS BETWEEN ETHEOSTOMATINE FISHES**



**by
CLARK HUBBS**

THE UNIVERSITY OF TEXAS AT AUSTIN

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Geographic Variations in Survival of Hybrids Between Etheostomatine Fishes

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Contents

	PAGE
ABSTRACT	5
INTRODUCTION	5
METHODS	8
SPECIES INVOLVED	11
HYBRID FERTILITY	23
INTERPOPULATION-INTRASPECIFIC HYBRIDIZATION	24
INTRAGENERIC ETHEOSTOMA HYBRIDS	31
INTERSPECIFIC HYBRIDS OF PRIMITIVE DARTERS	36
HYBRIDS BETWEEN PRIMITIVE AND ADVANCED DARTERS	42
DISCUSSION	54
<i>Egg Size</i>	54
<i>Hybrid Fertility</i>	57
<i>Patterns of Hybrid Survival</i>	58
<i>Phylogenetic Differentiation</i>	58
<i>Differences Between Reciprocals</i>	58
<i>Ecologic vs. Geographic Factors</i>	59
<i>Gametic Inhibition</i>	60
<i>Reinforcement</i>	62
<i>Introgression</i>	65
<i>Mutual Coexistence</i>	65
<i>Proximate Inhibition</i>	66
ACKNOWLEDGMENTS	68
LITERATURE CITED	68

List of Tables

	PAGE
1. Relative survival of interpopulation crosses of <i>Etheostoma spectabile</i>	25
2. Relative survival of interpopulation crosses of <i>Etheostoma lepidum</i>	26
3. Relative survival of interpopulation crosses of <i>Etheostoma caeruleum</i>	28

	PAGE
4. Relative survival of interpopulation crosses of <i>Hadropterus scierus</i>	28
5. Relative survival of interpopulation crosses of <i>Percina caprodes</i>	29
6. Relative survival of <i>Etheostoma spectabile</i> ♀ and <i>E. lepidum</i> ♂ hybrids	31
7. Relative survival of <i>Etheostoma lepidum</i> ♀ and <i>E. spectabile</i> ♂ hybrids	32
8. Survival of <i>Etheostoma lepidum</i> ♀ and <i>E. caeruleum</i> ♂ hybrids	34
9. Survival of <i>Etheostoma caeruleum</i> ♀ and <i>E. lepidum</i> ♂ hybrids	34
10. Survival of <i>Etheostoma caeruleum</i> ♀ × <i>E. spectabile</i> ♂ hybrids	35
11. Survival of <i>Etheostoma spectabile</i> ♀ × <i>E. caeruleum</i> ♂ hybrids	36
12. Survival of various intrageneric <i>Etheostoma</i> hybrids	37
13. Survival of <i>Hadropterus scierus</i> ♀ × <i>Percina caprodes</i> ♂ hybrids	40
14. Survival of <i>Percina caprodes</i> ♀ × <i>Hadropterus scierus</i> ♂ hybrids	40
15. Survival of hybrids using <i>H. shumardi</i> as a parental species	41
16. Relative survival of <i>Etheostoma spectabile</i> ♀ and <i>Percina caprodes</i> ♂ hybrids	43
17. Relative survival of <i>Percina caprodes</i> ♀ × <i>Etheostoma spectabile</i> ♂ hybrids	45
18. Relative survival of <i>Etheostoma lepidum</i> ♀ × <i>Percina caprodes</i> ♂ hybrids	47
19. Relative survival of <i>Percina caprodes</i> ♀ × <i>Etheostoma lepidum</i> ♂ hybrids	48
20. Average rankings of survival indices of greenthroat × logperch hybrids	49
21. Survival of various intergroup darter hybrids	49
22. Median index of success of various intergroup hybrids	53
23. Comparison of hybridization success with level of phylogenetic differentiation	60

Geographic Variations in Survival of Hybrids Between Etheostomatine Fishes

CLARK HUBBS*

Abstract

Comparative analyses of more than 500,000 darter eggs variously tested for fertility and viability from 1954 to 1965, and gathered from widely separated geographic locations, showed that darter hybrids were as viable as or more viable than the controls. Both reciprocals of 70 hybrid combinations were reared and more than 60 additional combinations had one reciprocal reared. Eggs were able to inhibit the activity of heterospecific sperm, especially after it had expended about one half its normal activity period; an inhibition obviously designed to reduce the effect of chance meeting of gametes in the water.

Several patterns of differential survival were noted, all associated with decreased hybridization potential in sympatry. Included were the first example of postmating reinforcement of isolation mechanisms, and an example of reinforcement being masked by more significant behavioral isolation. Several hybrid combinations had more viable hybrids in one reciprocal than in the other, always occurring where one parent had a high laboratory survival and the other was difficult to rear. All examples were maternally influenced.

Relative fecundity studies show that the darters in areas with many species have more and smaller eggs than equal sized females in the peripheral areas where few darter species occur.

Introduction

It has long been known that hybrids can be produced between relatively closely related species (Hertwig, 1936), and it is suggested that hybridization may affect selection of the parental taxa. Since such selection might be expected to affect interspecies' interactions, various explanations have been proposed for differences in responses to other species. Suggested causes have been arbitrarily classified into three major groups:

(1) The first is introgression as suggested by Anderson (1953) and others. That is, hybridization is selectively advantageous because it increases varia-

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tion. Therefore, those individuals that hybridize produce more vigorous offspring (the hybrids) than the others, and subsequent generations (of both species) contain relatively greater fractions of hybrid genotypes than might be expected otherwise. The hybridization results with a number of plant groups have been explained through introgression (Alston and Turner, 1963; Brown and Pratt, 1960; Hall, *et al.*, 1962; Haller, 1962; Raven, 1962; and numerous others); however, few examples are known of animals. Most studies such as those of Raney (1957) or McCarley (1954), provide evidence that the morphologic attributes of the parental taxa approach each other in sympatry but do not test the expected change in hybridization potential. If introgression is advantageous the sympatric populations should be more likely to produce hybrids than their allopatric relatives. Even Svardson's (1965) excellent account of introgression in *Coregonus* which shows the increased survival probability of F_1 hybrids does not demonstrate differences between the hybridization potential of sympatric and allopatric populations. Investigations designed to determine how often this happens are needed far more than further proof of morphologic similarity in sympatry.

(2) The second arbitrary classification of geographic variation in hybrid potential is reinforcement or character displacement (Dobzhansky, 1940). That is, hybridization is selectively disadvantageous to the parental species. Any individual that produced hybrids could not produce its own species with those gametes. Consequently those individuals would contribute less genetic material to subsequent generations. In contrast to introgression, this theory suggests that the hybrids do not contribute significant quantities of chromatin to subsequent generations and may displace one or both parental species ecologically. Therefore, hybrids between sympatric parents would be less likely to occur than would those between their allopatric counterparts, if they were placed together. Variations in the hybridization potential of several animal groups seem to fit the reinforcement model (Blair, 1955 and 1958; Hubbs and Delco, 1962; Koopman, 1950; Mecham, 1961; Sibley, 1961; and Vaurie, 1957). Similarly greater morphologic dissimilarity of sympatric species compared with allopatric species is in accord with the same model (Brown and Wilson, 1957, and citations).

(3) The third suggested result of hybridization interactions between related species is mutual coexistence outlined by J. A. Moore (1957). That is, hybridization does not play a significant role in speciation. According to this theory hybridization barriers are formed while the parental taxa are diverging to form distinct species in allopatry, and when sympatry occurs at a later date, hybridization is effectively impossible. The failure to produce intra-specific hybrids (Moore, 1946; Minamori, 1955; Duyvené de Wit, 1964) supports this theory.

One of the difficulties associated with evaluating some of the hybridization

results is ascertaining the species criteria of the respective authors. That is, Hall (1952) considers that *Juniperus ashei* and *J. virginiana* are allopatric species that exchange genetic material wherever their ranges contact. It is equally plausible to consider them morphologically distinct geographic races of the same species, and therefore the use of this example as introgression may be challenged. In contrast Minamori (1955) has shown that the two morphs of *Cobitis taenia* are unable to produce viable hybrids. Biologically the morphs are acting as distinct species, and if so they are not a valid example of hybridization inhibition within races of a single species.

Despite the occasional difficulty in ascertaining the phylogenetic differences between the taxa studied, all three of these interspecies interactions undoubtedly occur. It is also likely that the three theoretical models grade toward each other. That is, the selection toward introgression may occur between entities that previously had lost most of their potential to hybridize in allopatry, or that reinforcement may similarly act with previous existing reductions in hybridization potential. Likewise, the apparently contradicting introgression and reinforcement may differentially affect the same species pair, especially at different points of secondary contact. Therefore, a variety of patterns might be predicted.

Teleost fishes are among the animal groups best known for production of natural hybrids (C. L. Hubbs, 1955), and would be expected to show the effects of interspecies interactions through hybridization. The members of the subfamily Etheostominae previously have been shown to be amenable to hybridization experiments (Hubbs and Strawn, 1957a, c; Hubbs, 1959) and it is probable that any intrasubfamilial hybrid combination can be reared to mature size; however, hybrids between etheostomatines and related teleosts die before hatching (Hubbs, 1967), showing a correlation of hybrid potential with phylogeny. Although most or all etheostomatine hybrids can be produced, only one of the numerous natural hybrids (C. L. Hubbs, 1955) is between taxa representing the major subdivision of the subfamily (Hubbs and Laritz, 1961a). It is therefore apparent that hybrid inviability is only one of the many factors involved in preventing gene exchange. It is likely that habitat preference (Hubbs, *et al.*, 1953), breeding site and seasonal restrictions (Winn, 1958a), gametic compatability (Hubbs, 1959), mate preference (Hubbs and Martin, 1965), and others all mutually serve to maintain genetic stability.

Etheostomatine fishes are useful for studies of geographic variation of hybridization potential. The local populations can be distinguished morphologically (Strawn, 1961) so that the various races are likely to reflect the circumstances of that locality and not be extensively distorted by gene flow from distant populations subject to different selective factors. Adults are seldom used for bait or raised commercially so that human dispersal is nil.

Adults seem to be more or less sedentary. Although certain etheostomatines will breed in one environment and ripe individuals will move there only to move away between spawns, extensive collecting at one locality throughout the breeding season might depopulate that locality, indicating that adults do not move extensively during the breeding season. Developmental, probably free swimming larval, stages are likely to be the major source for dispersal. An over-worked collecting site will soon be populated with young fish, indicating that dispersal occurs during ontogeny. Likewise, there is some evidence that nonreproductive adults will move about. Most of the above notes on darter home ranges are based on observations of *E. lepidum* and *E. spectabile*; however, more limited observations on the other species are not discordant with the outline. Hubbs and Strawn (1957b) showed that one (and probably most) etheostomatine species breeds several times during a reproductive season. A long reproductive season permits numerous tests of hybridization.

Etheostomatines are also useful for hybridization experiments because they are relatively easily maintained in the laboratory (Strawn, 1955). They will spawn under a variety of circumstances and are not subject to a number of typical aquarium diseases; i.e., we have lost none to ich.

Methods

Eggs were stripped into enamel pans or glass finger bowls following techniques described by Strawn and Hubbs (1956). Each group of eggs was then placed in a location where temperatures were known to be relatively consistent. The eggs were examined daily, development recorded, dead eggs removed, and temperatures recorded. After completion of the experiment the temperatures were averaged and rounded to the nearest °C. for comparisons. The thermal survival curves were then smoothed by a running three-point average. Most of the thermal variations in the experiments were less than $\pm 1.0^{\circ}\text{C}$. The magnitude of thermal variation is known to affect the survival during etheostomatine ontogeny (Hubbs, 1964a); however, the variations during the experiments reported here were typically less extreme and equivalent among the tests so that comparisons of relative survival are considered valid.

The numbers of eggs and resulting larvae were carefully checked five times, providing survival figures for four intervals. The first counts were of the number of extruded eggs. The second counts were of the number of eggs containing embryos with pigmented eyes. This stage is easily recognized and apparently seldom if ever achieved by gynogenetic eggs (see Hubbs and Drewry, 1960, for a discussion of the problem). Many hybrid larvae were from parents with distinctly different larval stages; all such examples pos-

sessed morphologic features of both of the parental species showing that they were hybrids. At optimal temperatures the fraction of eggs containing embryos with pigmented eyes is thought to approximate the percent of fertilized mature eggs. As this figure varies widely between tests (probably because of variations in the efficiency of the investigator), further developmental success is based on the percentage of eyed eggs to achieve the appropriate stage. The third stage is hatching; most darters hatch at approximately the same stage of ontogeny after an interval approximately twice that required to develop pigmented eyes. Darter organogenesis is affected differentially by temperature, but no reversal in time of appearance of structures, such as those reported by Hayes, *et al.* (1953) for salmonids, has been noted. The fourth stage recorded was the number of young that completed prolarval life. Pro-larval life span is approximately equal to that preceding hatching, and is arbitrarily designated to be so. The last stage recorded was survival through the postlarval stage. Because this stage contains individuals from prolarval stages to small juveniles, its length was arbitrarily set as equal to the sum of prehatching and prolarval stages.

Survivals through four developmental stages, individually or in various combinations, are therefore available for comparisons. Similar results prevail in tests with large numbers of eggs so that each analysis undoubtedly reflects the same relative survival. Two figures are presented in this report. The first is the sum of percent fertilization, percentage of fertilized eggs to hatch, percentage of fertilized eggs to complete prolarval stages, and percentage of fertilized eggs to live through postlarval life. Greater emphasis is placed on early stages because a variety of unknown circumstances might affect survival in later stages. Fewer of these are effective during the early stages. Micro-organisms in rearing pans would be likely to increase after the experiments had been maintained for weeks. The second figure analyzed is the sum percentage of survival of the two stages of posthatching. This figure is considered to represent only hybrid survival, because maternal influence typically slows at gastrulation and is not likely to be preponderant beyond hatching.

The temperature range for thermal survival varies among species at a single locality (Hubbs, 1961a) and between different populations of a single species (Hubbs and Armstrong, 1962; Hubbs and Strawn, 1963). Therefore, comparisons of relative survival at a single temperature are hazardous. The data presented here are the sum of survivals at comparable temperatures. That is, comparisons are made only at those temperatures at which data are available for both the hybrid and the parental species. Most parental species had equivalent survival rates, and the survival rates of the hybrids were contrasted with the combined parental rates. When the parental survivals are very different from the hybrid survival, both relative figures are presented. In order to determine different effects of temperature on developmental

survival, most species were raised at a variety of temperatures. The ranges of survival are listed under the various species accounts.

The percentage of eggs fertilized with heterospecific sperm approximates that of the controls when the tests are made in a damp pan. Tests also have been carried out to determine the duration of sperm function in a large volume of water. There is a gradual reduction in sperm vitality with time and this reduction is greater in hybridization experiments than in the controls. The tests follow the techniques presented by Hubbs (1957, 1960, and 1961b). Briefly, the experiments were carried out by placing one gallon of aged Austin tap water, at $15 \pm 2^\circ\text{C}$. in an enamel pan. Approximately one pinhead of semen was stripped into the pan and the water stirred. After an interval timed by a stop watch, a set of eggs was scattered in the pan. The number of eggs developing pigmented eyes was considered to be the percent fertilized. The survival numbers were lumped by seconds (10.0–10.9 etc.) and then three-point running averages calculated. The resulting figures were compared with those of the paternal controls. Temperature differences of 5 to 10°C . were shown to change sperm vitality. Preliminary tests at 20°C . showed that sperm lost their fertilizing capacity after less time in the water than they did at 15°C .; however, no significant differences were noted within the temperature range utilized. Moreover, more than 90 percent of the tests were at $15^\circ \pm 0.5^\circ\text{C}$. Thus, if experimental circumstances were equivalent, the percentage of eggs to be fertilized would depend upon biological factors. Most of the extensively tested controls had more or less equivalent fertilization percentages up to about ten seconds delay and then a gradual decrease. It seems likely that experimental circumstances seriously affect the tests at less than ten seconds delay and biologic factors are more significant with longer delay. Therefore, the comparisons listed below are limited to those with more than ten seconds delay at $15 \pm 2^\circ\text{C}$.

Any differences in survival rates of hybrids and controls in experiments may be caused by a variety of extrinsic factors; therefore, statistical treatment has been conservative. Obviously, the genetic survival potential would vary among siblings, permitting differential survival within experiments. Superimposed on this intrinsic survival pattern are a number of extrinsic factors. If the biotic and/or chemical environment happens to be adverse, a greater fraction of fish die than would those in a favorable environment. Therefore, the survival of eggs is not necessarily independent. Moreover, if a series of fish are collected from an environment with reduced quantities of an essential nutrient, this adverse factor might affect all experiments based on the fish. Therefore, the survival of experiments is not necessarily independent. As a consequence, statistical comparisons usually are not presented; however, the 0.001 level is used in this paper as an index of significance in comparing survival of a single hybrid combination with those of its controls. The relative

survival of a variety of combinations is more likely to be independent and more normal levels of significance are used when contrasting relative success of different combinations.

Racial variations in egg sizes have been reported occasionally. Because the numbers of eggs available relate to the necessity to conserve eggs, and because size inversely reflects egg number, an analysis of variations in egg size should reflect reproductive potential. The eggs from a single female were permitted to water harden and five were measured by use of an ocular micrometer. The median size was recorded and these averaged. This technique is modified from that proposed by Clark (1925).

Species Involved

The listing of species follows that of Bailey and Gosline (1955) and the generic ranking follows that of Bailey (1951). The common names and geographic ranges were taken from G. A. Moore (1957). Semicolons separate river systems.

Hadropterus scierus Swain. The species ranges from the Guadalupe River System, Texas, to central Indiana. The stocks used came from the Guadalupe River at Gonzales and San Marcos River at various localities within three miles of San Marcos; the Colorado River and Onion Creek in Austin; the San Gabriel River at Georgetown and the Lampasas River four miles southwest of Belton; various tributaries to the Neches River in Rusk, Nacogdoches, Polk, and Tyler counties; and various tributaries to the Sabine River in Sabine County. The Guadalupe River stock has been named as a distinct subspecies (Hubbs, 1954), and Hubbs and Johnson (1961) showed that the females from the Guadalupe River had fewer and larger (1.78 mm.) eggs than those from the Colorado River (1.64 mm.). Brazos River System and Neches River System eggs average even smaller, being 1.58 mm. and 1.45 mm. respectively.

The dusky darter has been shown to have a narrow developmental temperature tolerance, primarily between 22° C. and 27° C. (Hubbs, 1961a). Additional experiments confirm the range and the relatively low survival (up to 25 percent) at any single temperature. Hubbs (1959) reported that five of eleven attempted intrafamilial hybrids based on *H. scierus* eggs had been reared through larval stages—*P. caprodes*, *E. radiosum*, *E. spectabile*, *E. caeruleum*, and *E. lepidum* being the successful paternal species. Subsequent tests show survival through larval stages using *H. shumardi*, *E. grahami*, *E. chlorosomum*, *E. tetrazona*, *E. punctulatum*, and *E. nianguae* sperm. One of the above, *E. chlorosomum*, had previously been tested unsuccessfully. Using less refined techniques, hybrids based on *E. proeliare*, *E. fonticola*, *E. microperca*, and *E. asprigene* sperm also have been reared. The first two had been tried unsuccessfully so that three failures are retained. Two additional fail-

ures have been recorded—*H. copelandi* and *E. zonale*—both based on one try. Of the five failures now on record, none has been tried more than three times. It is not surprising to have occasional failures of hybrids derived from the eggs of a species with up to 25 percent survival.

Hadropterus phoxocephalus (Nelson). This species ranges from Oklahoma and Arkansas to Minnesota and Pennsylvania. The specimens were obtained from the Big Maries River near Westphalia, Missouri. The eggs averaged approximately 1.21 mm.

The single attempt at a slenderhead darter control died before hatching. Eggs from one test using *E. caeruleum* sperm were successfully reared through the larval stages. Those based on *P. caprodes* and *E. tetrazona* died at the same stages as the maternal controls. Failure of two of three hybrids whose maternal control died is not unexpected. Using less refined techniques, hybrids based on *P. caprodes* sperm were reared, further indicating the high survival potential of hybrids.

Hadropterus evides (Jordan and Copeland). This species ranges from Arkansas to Iowa to New York. The specimens were obtained from Richland and War Eagle creeks, Washington County, Arkansas. The eggs averaged approximately 1.76 mm.

No controls have been attempted on this species and all three hybridization combinations were unsuccessful. The males were *P. caprodes*, *H. phoxocephalus*, and *E. lepidum*. This is among the least successful series of hybridization experiments available; however, the failures are based on minimal tests and related species are difficult to rear in the laboratory.

Hadropterus shumardi Girard. This species ranges from Canada to Texas. The specimens were obtained from the Guadalupe River at Gonzales. The eggs averaged 1.67 mm.

The river darter has not been reported previously to have been reared in the laboratory. Control survivals are low but occurred between 13° and 26° C. Successful survival of some hybrids through larval stages were those using *H. scierus*, *P. caprodes*, *E. spectabile*, *E. lepidum*, *E. euzona* sperm. Four combinations, all based on one test, were not successfully reared, *E. caeruleum*, *E. chlorosomum*, *E. proeliare*, and *A. vivax* providing the sperm. The failures of four of the combinations are not surprising because more than half of the control experiments also failed. The first two listed failures as well as hybrids with *E. radiosum*, *E. fonticola*, *E. punctulatum*, *E. asprigene*, and *E. gracile* sperm have been subsequently reared using less refined techniques.

Hadropterus copelandi (Jordan). This species ranges from Oklahoma to Canada. The stock of the channel darter came from the Little River near Nashoba, Oklahoma. The single control died after hatching as did the hybrids based on *P. caprodes* and *E. radiosum* sperm. The failures of the hybrids are not surprising because the maternal controls also failed. Using less refined

techniques hybrids have been reared using sperm of *E. radiosum*, *E. spectabile* and *E. asprigene*.

Percina caprodes (Rafinesque). This species ranges from Canada to Texas. The specimens were obtained from several localities in the Guadalupe River between Hunt and Waring, the Guadalupe River at Gonzales, and the San Marcos River within three miles of San Marcos; the Colorado River in Austin, Cummins Creek north of Fayetteville in Fayette County, Pedernales River in Hays County, Llano River at Junction, San Saba River at Menard and Ft. McKavett, and the S. Concho River four miles south of Christoval; San Gabriel River at Georgetown, Salado Creek at Salado, Lampasas River four miles southwest of Belton, the Brazos River in Falls County, Texas; an unnamed creek just north of Wilbarton, Oklahoma; Clear Creek and Muddy Fork near Savoy, Arkansas; the White River near Durham, Arkansas, Richland Creek and War Eagle Creek east of Fayetteville, Arkansas; and the Little and Big Maries rivers near Westphalia, Missouri.

Racial variation has been recorded for *P. caprodes* by a number of authors, and at least three subspecies are often recognized. All of the material here discussed is traditionally placed in the subspecies *carbonaria*. Within this group Hubbs and Strawn (1963) have shown racial differences in developmental tolerance, and Hubbs (1958b) has shown that females from the Colorado River in Travis County contain more eggs than females from the Guadalupe River in Kerr County. This is substantiated by studies of egg size. Kerr County eggs average 1.74 mm. and Travis County eggs average 1.63 mm. The difference could involve river systems or west longitude. The latter seems likely because western Colorado System eggs average 1.71 mm. and eastern Guadalupe system eggs average 1.60 mm. There seems little question that females collected from the edge of the Edwards Plateau have more and smaller eggs than those collected to the west in the middle of the plateau. Eggs from females from the Brazos system at the edge of the Plateau average 1.49 mm. Those from the Illinois system in Arkansas average 1.44, those from the White system in Arkansas average 1.59, and those from the Big Maries in Missouri average 1.35 mm. Winn (1958a) reported that a Michigan sample averaged 1.31 mm. The trend toward smaller eggs is, therefore, both west to east and south to north.

The *P. caprodes* variation is complicated by the presence of a second morph in Central Texas. It has been collected from the Guadalupe River six miles east of Kerrville, the Guadalupe River at Gonzales, the Colorado River in Austin, and the Pedernales River in Hays County. This morph differs from "typical" *P. caprodes* of Central Texas by having finer features; i.e., the color marks are thinner, the head structure is more delicate, the fin spines are less stout, etc. Similarly, the eggs are smaller, averaging 1.32 mm. A multitude of taxonomic questions concerning the two sympatric morphs of *P. caprodes* are

not resolved. It is, of course, possible that the differences reflect some variation in developmental circumstances; however, it is more probable that the morphs represent distinct species because intergrades have not been found, and they are sympatric over a rather large area. Which of the two morphs actually represents *P. caprodes* is difficult to determine. Traditionally the coarse morph has been called *P. caprodes*; however, the color patterns and egg size of the fine morph more closely resemble the attributes of Missouri specimens. In contrast, stocks from intermediate localities tend to form a cline between Missouri and the coarse morph of Central Texas. An additional complication is the allocation of the name *carbonaria*. Girard's (1859) description and Evermann and Kendall's (1894) discussion do not serve to determine which morph was used and the best distinguishing characters are not those that would be present on one hundred year-old specimens. The types are from the sympatric area. In this paper the coarse morph is called *P. caprodes* and the fine morph *P. species*.

Up to 25 percent of the logperch controls can be reared at temperatures between 22° C. and 25° C. Hubbs (1959) has shown that the eggs can be crossed successfully with *H. scierus*, *A. vivax*, *E. blennioides*, *E. radiosum*, *E. lepidum*, and *E. spectabile* sperm, and that the test with an *E. fonticola* male failed. Additional successful hybridization experiments with *P. caprodes* eggs occurred with *P. species*, *H. shumardi*, *E. tetrazona*, *E. grahami*, *E. caeruleum*, *E. punctulatum*, and *E. flabellare* males. Single unsuccessful tests were also made with *H. copelandi*, *H. phoxocephalus*, *E. zonale*, and *E. euzona* sperm. The five failures are not surprising considering that control eggs have a relatively low survival. Moreover, *E. fonticola*, *E. chlorosomum*, and *E. asprigene* sperm now have produced viable hybrids in subsequent less refined tests.

Up to 25 percent of the controls of *Percina* species develop at temperatures between 19° C. and 25° C. Successful hybridization has been carried out with *P. caprodes*, *H. scierus*, *H. shumardi*, *E. lepidum*, and *E. spectabile* males.

Ammocrypta vivax (Hay). This species ranges from Texas to Missouri. The stocks come from Horse Pen Creek west of Woodville, Texas. Hubbs (1959) reported failure of both hybrids and controls. The only subsequent tests on the southwestern sand darter are based only on males so that the results will be listed under the maternal parent.

Etheostoma chlorosomum (Hay). This species ranges from Minnesota to Texas. The stocks were obtained from Cummins Creek north of Fayetteville, Texas; various Neches River tributaries in Nacogdoches, Polk, Tyler, and Rusk counties; and Sabine River tributaries in Sabine County. The eggs averaged 1.05 mm. in diameter.

One of six control experiments with the bluntnose darter was successful. It was done at 26° C. Hubbs (1959) reported failure of the only attempted

hybrid with *E. lepidum* sperm. Successful hybridization is here noted using *E. radiosum*, *E. lepidum*, *E. spectabile*, and *H. scierus* males. The few tests with *E. gracile*, *H. shumardi*, and *P. caprodes* sperm all failed. Failure of three of seven combinations using eggs of a species in which one of six controls survived is not unexpected. Using less refined techniques, hybrids based on *E. nigrum*, *E. punctulatum*, *E. asprigene*, and *E. gracile* sperm have also been reared.

Etheostoma nigrum (Rafinesque). This species ranges from Oklahoma to Canada. The single male used came from the Big Maries River near Westphalia, Missouri. Hybrids have been reared based on *E. punctulatum*, *E. blennioides*, *E. radiosum*, and *E. lepidum* sperm after this manuscript was completed.

Etheostoma stigmaeum (Jordan). This species ranges from Oklahoma to Florida. The stocks were obtained from Clear Creek near Savoy, Arkansas; and the White River near Durham and Greenland, Arkansas. The Illinois System eggs averaged 1.07 mm. and the White System eggs 1.38 mm.

All four control experiments with the speckled darter failed. Hubbs (1959) reported no hybrids reared using males of five species with these eggs. Successful hybrids were subsequently produced using *E. juliae*, *E. caeruleum*, and *E. spectabile* males, and one more failure with *E. euzona* sperm was noted. Three successful and six unsuccessful combinations are not surprising using eggs of a species not yet reared experimentally. The successful combinations often were tried more times than the failures. Using less refined techniques hybrids with *E. nigrum* and *E. blennioides* males were reared.

Etheostoma tetrazona (Hubbs and Black). This species is found in Missouri River tributaries in Central Missouri. The stocks were obtained from the Big Maries River near Westphalia, Missouri. The eggs averaged 1.63 mm.

Up to 20 percent survival of Missouri saddled darters was noted between 18° C. and 28° C. No previous hybridization has been reported. Hybridization was successful when *E. caeruleum*, *E. spectabile*, *E. punctulatum*, *E. lepidum*, *E. juliae*, and *P. caprodes* males were used, and the one attempt with *E. stigmaeum* sperm failed. Again the survival of hybrids was equal to or greater than that of the controls. The subsequent rearing of hybrids based on sperm of *E. flabellare*, *E. euzona*, *E. zonale*, *E. blennioides*, and *E. radiosum*, is in accord with an hypothesis of hybrid vigor.

Etheostoma euzona (Hubbs and Black). This species is found in the White River system of Arkansas and Missouri and associated areas. The stocks were obtained from the Buffalo River, War Eagle Creek, and the White River near Durham, Arkansas.

The single control of the Arkansas saddled darter failed; however, it is probable that survival approximates that of its near relative, the Missouri saddled darter. Hubbs (1959) reported survival of hybrids with *E. caeruleum*

sperm. Additional hybrids were produced successfully with *E. lepidum* sperm, and the single test with *E. punctulatum* sperm failed. Two of three hybrid combinations were raised and the single control failed.

Etheostoma zonale (Cope). This species is found from Arkansas to Georgia to the Lake Michigan drainage. The stocks were obtained from Muddy Fork near Savoy, and Clear Creek at Savoy and Johnson, Arkansas; the White River at Durham and Greenland and a tributary, Richland Creek; and the Big Maries River near Westphalia, Missouri. The Illinois and White system eggs averaged 1.56 mm., and those from the Big Maries were smaller, averaging 1.42 mm.

Control experiments on the banded darter have been tried fourteen times. None was reared through the larval stages. The best success was at 26° C. and 27° C. One of six subsequent less refined control experiments was successful at 20° C. It was the result of a Big Maries × Richland Creek interpopulation test. Hubbs (1959) reported rearing hybrids with *E. caeruleum* males and failures with *E. euzona* and *H. nigrofasciatus* sperm. Additional successful tests were carried out with *E. spectabile*, *E. lepidum*, *E. stigmaeum*, *E. blennioides*, *E. tetrazona*, *E. punctulatum*, *E. nigrum*, and *P. caprodes* males. Two additional failures were noted, all based on three or fewer tests with *E. juliae* and *H. phoxocephalus* sperm. Successful rearing of nine of 13 combinations must be considered to be high survival because the intrapopulation maternal controls have not yet been reared. Moreover, the maximum number of tests with an unsuccessful hybrid combination was three, and all fourteen intrapopulation control tests failed. Subsequent experiments have resulted in successful rearing of hybrids with *H. copelandi*, *E. flabellare*, and *E. radiosum* sperm.

Etheostoma blennioides (Rafinesque). This species ranges from the Ozarks to the Great Lakes. The specimens were from Clear Creek at Johnson's and Greathouse Springs north of Fayetteville, Muddy Fork near Savoy, Arkansas; the White River at Greenland and Durham, Brush Creek east of Fayetteville, Arkansas; and the Big Maries River at Westphalia, Missouri. The Illinois (1.81) and the White River (1.76) eggs were larger than those from the Big Maries (1.52). Northern eggs are much larger. Winn (1958a) obtained an average of 1.85 mm. for Michigan eggs; and Fahy (1954) obtained, preserved (and presumably shrunk) mature eggs between 1.83 and 1.89 mm. from Salmon Creek, New York, females.

Only six control experiments have been run on the greenside darter, and one at 24° C. was successful. Fahy (1954) showed that a New York population spawned from 13° C. to 22° C. Apparently there is some geographic variation in temperature adaptations. The two controls run at 14 and 16° C. failed. No previous hybridization has been recorded; and success was attained with *E. spectabile*, *E. lepidum*, *E. punctulatum*, *E. caeruleum*, and *P.*

caprodes sperm. No survival occurred in experiments with *E. tetrazona* males. The failure of one of six combinations is not surprising considering that one of six control experiments was successful and that subsequent tests with males of six other species, *E. chlorosomum*, *E. nigrum*, *E. stigmaeum*, *E. zonale*, *E. tetrazona*, and *E. flabellare*, all succeeded.

Etheostoma nianguae (Gilbert and Meek). This species is restricted to central Missouri. The fish were obtained from the Big Maries River near Westphalia, Missouri.

All individuals of the single control test on the Niangua darter died before completion of larval stages. Hybridization was successful with *E. blennioides* and *P. caprodes* sperm, and unsuccessful with *E. zonale* males. Hybrid survival compares favorably with control survival.

Etheostoma juliae (Meek). This species occupies the White and James River systems in Arkansas and Missouri. The stocks were obtained from the Buffalo River, War Eagle Creek, and the White River from near Durham, Arkansas. The eggs averaged 1.68 mm. in diameter.

The yolk darter has been reared twice in seven attempts, once at 20° C. and once at 28° C. The thermal range for developmental survival is unknown but is likely to have an upper limit near 30° C. and a lower limit near 20° C. No hybridization has been reported previously, and success was attained using *E. caeruleum*, *E. spectabile*, *E. tetrazona*, *E. lepidum*, and *E. punctulatum* sperm. No survival through the larval stages occurred in the tests with *E. zonale* and *P. caprodes* males. The survival of five of seven hybrid combinations contrasts favorably with the survival of two of seven controls.

Etheostoma punctulatum (Agassiz). This species is restricted to Ozark streams. Most fish were collected from Little Wildcat Creek and Greathouse Spring north of Fayetteville, Arkansas. Others were taken from Brush Creek near its mouth into the White River and Muddy Fork of the Illinois near Savoy. The Greathouse females had eggs averaging 1.50 mm.

The stippled darter has been reared from 19° C. to 24° C. Survivals may exceed 75 percent although the sample sizes are often minimal. No hybridization has been reported previously, and successes are here recorded with *E. spectabile*, *E. lepidum*, *E. caeruleum*, and *E. tetrazona* sperm; and a single experiment with one *E. juliae* male failed. Hybrid survival again compares favorably with that of the maternal controls. Using less refined techniques, hybrids based on sperm of *E. zonale* and *E. blennioides* have also been reared.

Etheostoma asprigene (Forbes). Occurs in the Mississippi lowlands. The stocks were obtained from Gibbons Creek 4 miles west of Douglass, Nacogdoches County, Texas. No controls were attempted in the tests, all of which were done with the less refined techniques. Successful hybrids were produced with *E. blennioides*, *E. radiosum*, *E. caeruleum*, *E. spectabile*, and *E. gracile* sperm.

Etheostoma radiosum (Hubbs and Black) occurs in the Red River tributaries in Oklahoma, Arkansas, and Texas. The stocks were obtained from the Little River and its tributaries in Pushmataha County, Oklahoma; Neches River tributaries in Nacogdoches County; and Sabine River tributaries in Sabine County, Texas.

Up to 50 percent of the controls of the orangebelly darter can be reared at temperatures between 19° C. and 23° C. Hubbs (1959) reported hybrid survival through the larval stages with males of *E. spectabile*, *E. lepidum*, *H. scierus*, *P. caprodes*, and *A. vivax*, and a failure with *E. proeliare* sperm. Additional hybrids reared were fertilized by *H. copelandi* sperm, and the single test with *E. chlorosomum* sperm failed. The failures of the two combinations are not considered of major importance because only a single test was run with each of the species in which males produce minimal quantities of sperm. Moreover, one of them, *E. chlorosomum*, has subsequently produced viable hybrids as have males of *H. shumardi*, *P. species*, *E. stigmaeum*, *E. euzona*, *E. zonale*, *E. juliae*, *E. gracile*, *E. microperca*, *E. nigrum*, *E. tetrazona*, *E. blennioides*, *E. punctulatum*, *E. caeruleum*, and *E. whipplei*. The reciprocal of the last has also been reared.

Etheostoma caeruleum (Storer). This species ranges from the Ozarks to Canada. The stocks were obtained from the Buffalo River, the White River at Greenland, Durham, and its tributaries, Richland, Brush, and War Eagle creeks north and east of Fayetteville, Arkansas; and the Little Maries River, Big Maries River, and Loose Creek near Westphalia, Missouri. The White River eggs averaged 1.78 mm. (Brush), 1.79 mm. (Greenland), and 1.82 mm. (Durham). The Big Maries eggs were somewhat smaller, averaging 1.72 mm. The difference is supported by the much smaller average of the small Little Maries sample (1.47 mm.). Winn (1958a) reported that small females (yearlings) lay smaller (1.56 mm.) eggs than do larger, two year-old females (1.78 mm.); however, his sample size was small. Only occasionally have small, ripe eggs been noted in these experiments. All have involved small females, but more than 90 percent of the small females contain normal sized eggs.

The rainbow darter has been reared at temperatures between 11° C. and 29° C. Survivals were up to 50 percent, showing that this is a good laboratory animal. Hubbs (1959) reported survival of *E. caeruleum* eggs fertilized with *E. lepidum*, *E. euzona*, *E. juliae*, and *P. caprodes* sperm, and failures of one and two tests with *E. fonticola* and *H. scierus* sperm. Additional successfully reared hybrids are with *E. spectabile*, *E. tetrazona*, *E. zonale*, *E. punctulatum*, *E. flabellare*, *E. stigmaeum*, *E. nianguae*, *H. shumardi*, and *H. phoxocephalus* males. A single test with *E. blennioides* sperm failed before completion of larval stages. Only three of 16 combinations failed, and the three were based on a total of four tests. Again hybrid survival compares favorably with that

of the controls. Moreover, two of the three combinations that failed, *H. scierus* and *E. blennioides*, have been subsequently reared as have those with males of *A. vivax*, *E. chlorosomum*, *E. radiosum*, and *E. microperca*.

Etheostoma spectabile (Agassiz). This species ranges from Texas to Michigan. The stocks were obtained from the Guadalupe River just west of Mo Ranch, at Hunt, west of Ingram, five miles west of Kerrville, three miles east of Comfort, and near Kendalia, the San Marcos River, within four miles of San Marcos, and the Blanco River five miles east of Blanco; Cole Creek, Gillespie County, the Llano River at Junction, the San Saba River at Ft. McKavett, the South Concho River four miles south of Christoval, and the Colorado River in Austin; the San Gabriel River at Georgetown, Berry Creek three miles east of Georgetown, Salado Creek at Salado, the Lampasas River five miles south of Belton, and the Brazos River in Falls County; an unnamed creek north of Wilborton, Oklahoma; Mountain Creek, Franklin County, Arkansas; Muddy Fork and Clear Creek near Savoy, Greathouse Springs and Little Wildcat Creek near Fayetteville, and Clear Creek near Johnson, Arkansas; the White River near Winslow, Durham, and Greenland, and Brush Creek east of Fayetteville, Arkansas; and The Big Maries and Loose Creek near Westphalia, Missouri. The Guadalupe River eggs tend to be the largest, averaging 1.48 mm. to 1.57 mm. for four samples with ten or more females studied. The Kerr County samples are distinctly larger, averaging 1.52, 1.55 and 1.57 mm., than the San Marcos sample, averaging 1.48 mm. The Colorado system eggs tend to be slightly smaller, ranging between 1.43 and 1.52 mm. in diameter. The lower figure is for Austin and the upper for Junction samples. Similar to the results of *P. caprodes*, the western samples are invariably larger than those from the eastern edge of the Edwards Plateau. The Brazos samples, all from along the edge of the Plateau, average 1.46 mm. (Georgetown), 1.41 mm. (Salado), and 1.36 mm. (Lampasas) in diameter. The Illinois System eggs are relatively small, averaging 1.31 to 1.41 mm. in diameter. White River eggs are slightly larger, both samples based on ten or more females averaging 1.46 mm., the small sample from Brush Creek deviates by being merely 1.36 mm. The Missouri eggs are the smallest, the large sample from Loose Creek averaging 1.22 mm. and that from the Big Maries, 1.32 mm. Winn (1958a) reported small eggs (1.24 mm.) from Michigan. There is a distinctly smaller average egg size in northern samples. Apparently a north-south as well as an east-west cline prevails.

Hubbs and Armstrong (1952) have shown that northern eggs and larvae tend to survive better at warmer temperatures than do southern samples. They suggested that the difference was due to rapid seasonal warming in the north and similar thermally induced termination to the reproductive season. The larger samples now available substantiate the hypothesis. The survival until hatching is nearly equivalent, whereas the maximum temperature for

optimal postlarval survival is 26° C. to 27° C. in Texas populations and near 30° C. for stocks obtained in Loose Creek.

The orangethroat darter adapts well to laboratory conditions. Up to 75 percent of fertilized eggs at temperatures between 10° C. and 27° C. survive through the larval stages (Hubbs 1961a). Hubbs (1959) reported survival through larval stages of eggs fertilized with *E. lepidum*, *E. grahami*, *E. caeruleum*, *E. gracile*, *E. parvipinne*, *E. blennioides*, *E. fonticola*, *H. scierus*, and *P. caprodes* sperm, and failure of the single experiment using *E. radiosum* sperm. Additional hybrid combinations are with *E. radiosum*, *E. zonale*, *E. stigmaeum*, *E. flabellare*, *E. tetrazona*, *E. euzona*, *E. punctulatum*, *E. juliae*, *E. proeliare*, *H. phoxocephalus*, *H. shumardi*, *H. copelandi*, and *Percina* species males. The eggs of this good laboratory animal have been exposed to sperm of 22 other members of the same family and in all combinations individuals have passed through the larval stages. Occasional examples have failed as have some controls, and none can be considered to indicate hybrid inviability. Three additional combinations have subsequently been reared based on sperm of *E. microperca*, *E. whipplei*, *E. chlorosomum*, and *E. nigrum*.

Etheostoma grahami (Girard). This species is found in clear spring-fed tributaries of the Rio Grande. The specimens were obtained from Dolan Creek and San Felipe Creek in Del Rio.

The Rio Grande darter has been successfully reared (Strawn, 1961), but controls have not been attempted in this investigation. Hubbs (1959) reported one successful hybrid combination with *E. spectabile* sperm and a failure with *P. caprodes* sperm. A single test with *E. radiosum* has also failed. The two failures are based on a total of six fertilized eggs and are not to be considered to demonstrate hybrid inviability. Moreover, hybrids with *P. caprodes* males have subsequently been reared as have hybrids based on *H. scierus*, *H. shumardi*, *E. caeruleum*, and *E. microperca* sperm.

Etheostoma lepidum (Baird and Girard). This species occupies clear spring-fed waters of the Nueces, Guadalupe, and Colorado river systems. The stocks were obtained from the Nueces River five miles south of Camp Wood, and at Barksdale, the West Frio River and Kent Creek at their junction, and the Frio River at Leakey and Garner Park; the South Guadalupe River at Lynxhaven approximately nine miles west of Hunt, the North Guadalupe River at a spring one half mile west of Mo Ranch, the head spring and associated concrete ditches supplying the State Fish Hatchery at Mountain Home on Johnson Creek, the Guadalupe River at Hunt, three miles west of Ingram, five miles east of Kerrville, and three miles east of Comfort; the Colorado River and tributaries in Austin, the Llano River at Junction, the San Saba River at Ft. McKavett, and the S. Concho River four miles south of Christoval.

Hubbs and Delco (1960) reported that Nueces River females had fewer and larger eggs than any other population of *E. lepidum* analyzed. They also reported that Frio River females had relatively numerous (= small) eggs. Additional information shows that the apparently large numbers of Frio River eggs was erroneous, probably based on a small sample size—17 females—because the much more extensive Garner Park sample here reported—44 females—have egg sizes nearly identical with those from the Nueces System. The average egg sizes for samples with more than 10 females are 1.52 mm. (Garner Park), 1.53 mm. (Barksdale), and 1.56 mm. (Camp Wood). The egg size varies among the Guadalupe and Colorado populations with ten or more females analyzed, averaging 1.30 mm. nine miles west of Hunt, 1.34 mm. at Hunt, 1.39 mm. one half mile west of Mo Ranch, 1.31 mm. at the Mountain Home State Fish Hatchery, 1.34 mm. at Austin, 1.38 mm. at Junction, 1.28 mm. at Ft. McKavett, and 1.30 mm. four miles south of Christoval. The samples closer geographically to the Nueces system are no more likely to have large eggs than are those more distant.

This species can be reared at temperatures between 11° C. and 27° C. with survivals up to 80 percent (Hubbs, 1961a) based on the sample from the South Concho River, and subsequent experiments have extended the range to 8° C. to 29° C. The Nueces River sample deviates slightly by having its survival between 7° C. and 28° C. Hubbs (1959) reported that eggs of this species were successfully crossed with sperm of *E. spectabile*, *E. radiosum*, *H. scierus*, and *P. caprodes*. In addition the following males have successfully produced viable postlarvae with *E. lepidum* eggs: *E. chlorosomum*, *E. gracile*, *E. punctulatum*, *E. caeruleum*, *E. juliae*, *E. tetrazona*, *E. nianguae*, *E. blennioides*, *P. species*, *H. shumardi*, and *H. copelandi*. Single tests, each with *E. nigrum* and *E. proeliare* sperm failed. The latter has subsequently been reared as have hybrids with *E. zonale* and *E. asprigene* males.

Etheostoma flabellare (Rafinesque). This species is found from the Ozarks to Canada. The specimens were collected from Little Wildcat Creek, Great-house Spring, and Clear Creek at Johnson, all north of Fayetteville, Arkansas; and from Big Maries River and Loose Creek near Westphalia, Missouri. The Loose Creek eggs average 2.06 mm. and those from the Big Maries 2.23 mm. Comparable sized eggs (2.3 mm. and 2.22 mm.) were noted by Lake (1936) and Winn (1958a) for New York and Michigan populations respectively.

This species was not successfully reared in these experiments. The large eggs are difficult to expel without damage, and sperm quantities are minimal. The successful combinations are those with *E. spectabile*, *E. lepidum*, *E. caeruleum*, *E. tetrazona*, *E. punctulatum*, and *P. caprodes* males; and the failures with *E. zonale*, *E. blennioides*, *E. juliae*, and *H. phoxocephalus* males. Six out of ten hybrid combinations were successful despite the failures of the controls. Most of the unsuccessful combinations (including the controls) were

attempted fewer times than the successful ones. Moreover, the first two listed failures have been reared subsequently using other techniques.

Etheostoma gracile (Girard). This species ranges from Texas to the Great Lakes. The specimens were from Cummins Creek north of Fayetteville, Texas, Brazos River in Falls County and a tributary in Brazos County, and in Hardin County, Texas. The eggs averaged 1.01 mm.

This darter has not been reared using this technique, although naturally laid eggs have been reared. No hybrid combinations have previously been recorded. The hybrids have been successfully reared using *E. lepidum* and *E. spectabile* males, while the two tests with *E. punctulatum* males failed. The failure is not surprising considering the failure of the controls.

Etheostoma proeliare (Hay). This species ranges from Texas to Illinois. The specimens were obtained from Cummins Creek, Fayette County, Texas; various tributaries of the Neches River in Polk and Tyler counties.

This darter has not been reared in these experiments. A relatively large fraction of naturally laid eggs have been raised by other techniques. Hubbs (1959) reported that two males, *E. radiosum* and *A. vivax*, had produced hybrids that survived through the larval stages and that four combinations, with *E. stigmaeum*, *E. gracile*, *H. scierus*, and *H. nigrofasciatus*, did not. Four more tests failed using *E. lepidum*, *E. spectabile*, *H. shumardi*, and *P. caprodes* sperm. Failure of eight out of ten hybrid combinations is not as discordant as appears at first glance because maternal controls all failed under comparable circumstances. Moreover, the first listed new unsuccessful combination has been subsequently reared as have hybrids with *E. microperca*.

Etheostoma fonticola (Jordan and Gilbert). This species is found only in Central Texas. Stocks were obtained from the San Marcos River in San Marcos and Comal Creek in New Braunfels.

This species has been raised at temperatures between 20° C. and 24° C. The fertilization rate is exceedingly low with 24 fertilized eggs present in 57 control experiments, and only ten of these were reared. Hubbs (1959) reported success of hybrids with *E. spectabile*, *H. scierus*, and *P. caprodes* males; and failures with *E. lepidum*, *E. caeruleum*, *E. euzona*, and *E. juliae* sperm. No additional tests have been run, and the low hybrid survival is not surprising considering the exceedingly low survival of the controls. Since this manuscript was finished hybrids with *E. asprigene*, *E. radiosum*, and *E. microperca* have been reared.

Etheostoma microperca Jordan and Gilbert occurs in the upper Mississippi Valley. The stocks used in less refined experiments came from Little Spring Creek, Mayes County, Oklahoma. Hybrids were successfully reared based on sperm of *E. caeruleum* and *E. spectabile*.

Previously it has been concluded that any darter hybrid combination can be reared if technique problems are solved. A summarization of the successful

combinations supports this hypothesis. Fourteen of the 25 (56 percent) controls have been successfully reared; 92 of 144 (69 percent) of the within-group (intrageneric hybrids or those between *Hadropterus* and *Percina*, two closely related genera) combinations have been successfully reared; and 53 of 85 (62 percent) between-group hybrids have been successfully reared. This crude summarization clearly shows that darter hybrids are no more difficult to rear than are the controls.

Some darters are much more easily reared than are others. It is very apparent that the relative survival of the parental types is much more significant to darter hybrid survival than is the degree of phylogenetic relationship. The within-group hybrid combinations in which both parents have been successfully reared have been successful in 85 percent of the combinations, 59 percent of the combinations in which only one parent has been reared under comparable conditions have been successful, and only 20 percent of those in which neither parental type has been reared. Comparable figures for intergroup hybrids are 80, 53, and 0 percent. The differences in survival percentage associated with parental laboratory success are far greater than those associated with phylogenetic differentiation. There is a slight indication of a phylogenetic effect in that all intergroup combination survival figures are lower than comparable intragroup figures. None of the differences are of statistical significance, but may indicate an effect of evolutionary differentiation on hybrid survival.

The above calculations are only rough approximations because a combination tried a single time may have failed by chance alone. For instance, the single *E. euzona* control failed and less than 10 percent of the *E. fonticola* controls were successful. Obviously the latter is difficult to rear; and most of its hybrid combinations failed, yet the species has been arbitrarily categorized as successful. *Etheostoma euzona* might be expected to have a laboratory survival approximating that of its allopatric sibling, *E. tetrazona*, and would therefore be far more successful in the laboratory than *E. fonticola*. The relatively large number of reared hybrid combinations with *E. euzona* supports this hypothesis.

Eighteen of the combinations that failed were subsequently reared in less refined experiments that were primarily designed to obtain stocks for hybrid fertility tests. The high frequency of success with previous failures was in part based on experience in avoiding errors and on extra effort to remove failures.

Hybrid Fertility

Hubbs (1958a) reported that female orangethroat-greenthroat darter hybrids were fertile, and that the males were sterile. A number of putative hybrids that have been obtained in the field were also tested for fertility; no

males produced visible milt, and all eggs exposed to "stripped" hybrid males failed to develop. Hybrid females often produced eggs, and two from Junction produced eggs that averaged 1.43 mm. in diameter.

A few laboratory *E. grahami* San Felipe \times *E. spectabile* Lampasas have been reared to adult size. Eggs from these hybrids have been fertilized by sperm of *E. spectabile*, *E. lepidum*, *P. caprodes*, and *H. scierus*. The males seem to be sterile and produce no visible milt. Eggs of five hybrid females were tested with "stripped" hybrid males. None was fertilized. Only four of 18 sets of eggs tested against other species also failed, showing that the failures did not involve the eggs.

Hybrids between the *E. grahami* San Felipe and *E. lepidum* West Frio River are fertile, and females can be back-crossed to their parental species. Similarly, *E. lepidum* Ft. McKavett and *E. lepidum* West Frio River hybrids can produce F_2 's (all extracted from Strawn, 1961). Apparently *E. lepidum* and *E. grahami* are mutually interfertile and when tested for hybrid fertility with *E. spectabile* result in fertile female and sterile male F_1 's.

Lindner (1958) presented evidence that hybrid females of *E. radiosum* \times *E. spectabile* (reciprocal not stated) produce apparently normal eggs and that a male did not father young when isolated with a ripe *E. spectabile* female. Apparently darter hybrid fertility is most likely in females.

Interpopulation-Intraspecific Hybridization

The relative success of allopatric vs. sympatric hybrids may result from selection relative to the interaction of the two species, or it may result from differential adaptation to the specific environments. If the latter is exceedingly important it should affect the intraspecific hybrids which would then provide a type of control for the interspecific hybridization experiments. As shown below, intraspecific hybrids are often hererotic; however, inhibition is more common than in interspecific hybrids.

Etheostoma spectabile. A large number of hybrid survival tests have been carried out, contrasting different populations of the orangethroat darter (Table 1). More than 1,000 eggs were used in most of the comparisons, with the exception of Loose Creek \times White River, and White River \times Guadalupe River. In general the relative success of hybrid development is high with only two readings below 95 (both in total comparisons) and 21 over 105. Neither of the low readings is repeated in the other method of analyzing the data so that they may be considered to be of dubious significance, whereas ten of the high readings were repeated. Therefore, heterosis occurs frequently in interpopulations crosses.

The results of the time lapse fertilization experiments are distinctly different. Half of the figures are low, the other half high. The extreme variation in

TABLE 1

Relative survival of interpopulation crosses of Etheostoma spectabile. A figure of one hundred (100) would be equal to that of control (intrapopulation) experiments. Crosses involving a single river system are based on parents from two localities within that system and a least five miles apart.

	White R.	Illinois R.	Brazos R.	Colorado R.	Guadalupe R.
		(Posthatch)			
Loose Creek	100.7	134.5			
White R.		135.1		115.1	235.5
Illinois R.			144.4	149.6	112.8
Brazos R.			124.6	125.6	116.6
Colorado R.				117.6	97.0
Guadalupe R.					101.7
		(Total)			
Loose Cr.	95.4	108.7			
White R.		115.8		101.8	153.9
Illinois R.			122.1	118.7	120.2
Brazos R.			118.3	109.7	107.6
Colorado R.				109.3	94.4
Guadalupe R.					92.9
		(Time Lapse Fertilization)			
Loose Cr.	27.8	203.4			
White R.		137.0		190.9	
Brazos R.			58.9	35.8	92.8
Colorado R.					173.1

relative success may involve the somewhat smaller samples available, but is also probably due in part to differential selective factors. The relative fertilization success is not likely to be enhanced by heterotic phenomena. Moreover, time lapse fertilization is somewhat of a premating isolation mechanism and therefore likely to reflect incipient isolation. The patterns available do not provide data to show intraspecific subgroupings because they are inconsistent. For instance, the White River and Loose Creek systems have similar fish; however, they have an extremely low fertilization rate. On the other hand they both seem to be highly compatible with Illinois stocks. If they were different, the results with Illinois fishes should also differ. The figures do indicate, however, that some of the low successes of the hybrids' survival tests may be artifacts because one of the most successful fertilization test series is between the same populations that did not show heterosis (Guadalupe and Colorado).

Hubbs (1960) reported that Brazos River sperm was more vigorous than that of any other orangethroat darter population. It is likely that this involves

selective phenomena because no other species with which orangethroat darters might exchange genes is present in the appropriate segment of the Brazos. All of the time lapse experiments confirm this conclusion. Tests at comparable time delays show that Brazos River controls do better than those of the other stream systems. A summary of the results between six and 20 seconds' delay shows the fertilization rate of the Brazos controls to be one and one half times that of the White controls (the second best).

Etheostoma lepidum. All of the tests are based on large samples so that deviations from the expected index of 100 are statistically significant. The relative survival of the "interpopulation" hybrids is notably lower than that of the *E. spectabile* complex. Except for those based on the San Saba stocks (see below for an analysis of the San Saba results) only three figures are above 105, and five are below 95 (Table 2). The low figures involve three combinations—different populations within the Guadalupe System, and interstream system hybrids involving the Nueces System. The reason for low survival of hybrids from within the Guadalupe System is not readily apparent. Those interstream system results involving the Nueces System population are thought to show incipient allopatric speciation.

Time lapse fertilization studies indicate extensive speciation. Only the within-Colorado and within-Nueces comparisons even approach equality with the controls. Because large samples were used for all but the within-Nueces tests the reduced survivals are highly significant statistically. Therefore, the greenthroat darter seems to be in the process of dividing into at

TABLE 2
Relative survival of interpopulation crosses of Etheostoma lepidum.
Other circumstances as described in Table 1.

	Colorado R.	Guadalupe R.	Nueces R.
(Posthatch)			
Colorado R.	116.2	106.3	85.6
Guadalupe R.		81.3	77.8
Nueces R.			99.6
San Saba R.	151.2		119.6
(Total)			
Colorado R.	106.1	102.4	96.0
Guadalupe R.		89.7	84.8
Nueces R.			96.2
San Saba R.	115.6		106.4
(Time Lapse Fertilization)			
Colorado R.	94.4	73.0	68.4
Guadalupe R.		60.0	67.2
Nueces R.			135.7

least three taxa, one in the Nueces, one in the Colorado, and one or more in the Guadalupe.

The Nueces River stock of *E. lepidum* resembles the Brazos River stock *E. spectabile* in its inability to exchange genes with any sympatric species. The two stocks differ greatly in relative vitality of the sperm, the Nueces populations having less vigorous sperm than comparable populations inhabiting other stream systems. The reduced vitality is in distinct contrast with the high sperm viability of Brazos River *E. spectabile*. Large eggs and reduced egg complements of Nueces system females (Hubbs and Delco, 1960) may be responsible for reduced vitality of sperm in males from that stream system.

The survival of fishes from the San Saba River is distinctly lower than that of comparable fishes from any other system. The summed survivals are 79 and 91 percent of the other Colorado system localities. Vitality is most reduced during postlarval development, and survival of fertilized eggs through the larval stages is 40 to 50 percent of that of the other localities within the Colorado System. The low survival of the San Saba population may be due to genetic or environmental circumstances; more probably, environmental circumstances are involved because *P. caprodes* controls from the San Saba River also show low survival. Another set of circumstances seems to indicate this. Extensive pecan groves are present at the headwaters and sprayed with insecticides in spring months. Two fish kills have occurred in the last decade. Survival of San Saba eggs is highest in the spring just before the recommended spraying interval and lowest in the fall after the spray interval. Insecticides are known to adversely affect bird reproduction (Bernard, 1963). These factors indicate but do not prove an external environmental effect causing the low survival of San Saba stocks. Whether the assumption is valid or not, the low survival necessitates treating the post-fertilization data separately.

The adverse effect seems to occur in both reciprocals of hybrid tests. That is, survival of intrastream system hybrids through larval stages using San Saba stocks is below that of the non-San Saba controls, regardless of which reciprocal is used. Similar within-Colorado system interpopulation hybrids are invariably heterotic. The prehatching figures are more or less equivalent, indicating that the mortality is posthatching. Despite reduced vitality of the San Saba hybrids, the survival data presented on Table 2 are above 100, because comparisons were based on survival of both parental types. The exceedingly low San Saba figures more than compensate for the somewhat reduced hybrid survival. Despite problems in evaluating survival of San Saba hybrids, the relative survivals can be used. Hybrids with Nueces stocks invariably have distinctly lower survival than do those with other Colorado stocks. The survival reductions approximate those of other (high survival) Colorado stocks crossed with Nueces River stocks.

Etheostoma caeruleum. Relatively few tests were carried out on interpopulation survival of rainbow darters. Survivals were either consistently high, within-White, or low, White River \times Loose Creek (Table 3). The former are more likely to be valid because of the much larger number of experiments. Moreover, the single time lapse fertilization experiment, Loose Creek \times White River, was more successful than the controls.

Hadropterus scierus. The number of tests run on relative survival of interpopulation hybrids of blackside darters was rather limited. Three comparisons were below 95, and six above 105, indicating an overall pattern of heterosity (Table 4). The only apparent inhibition was within the Neches River System, and the analyses were based on too few eggs from too many different creeks to base any definite conclusions on the results. The Guadalupe \times Brazos experiments were contradictory by being low and high simultaneously, depending on which stage was studied. The extensive Colorado River experiments were strongly heterotic.

TABLE 3
Relative survival of interpopulation crosses of Etheostoma caeruleum.
Other circumstances as described in Table 1.

	Loose Cr.	White R.
	(Posthatch)	
White R.	91.4	164.5
	(Total)	
White R.	91.6	154.5
	(Time Lapse Fertilization)	
White R.	661.1	0.0

TABLE 4
Relative survival of interpopulation crosses of Hadropterus scierus.
Other circumstances as described in Table 1.

	Neches R.	Brazos R.	Colorado R.	Guadalupe R.
		(Posthatch)		
Neches R.	36.0		325.9	
Guadalupe R.		114.4	105.3	98.3
		(Total)		
Neches R.	84.5		166.6	
Guadalupe R.		80.0	108.4	122.1
		(Time Lapse Fertilization)		
Neches R.			178.5	
Guadalupe R.		296.1	34.9	

Time lapse experiments were equally few but also indicate an overall heterosis. The low figure for Colorado \times Guadalupe hybrids may be due to chance or may indicate that the morphologic distinction between the Guadalupe and Colorado fish has an associated isolation mechanism.

Percina caprodes. Intraspecific hybrid survival experiments on logperch were mostly more successful than the controls (Table 5). Only four tests were below 95, and 14 were above 105. Only one of the low figures was repeated on both analyses, and that (Brazos \times Oklahoma) was based on 55 eggs. Two of the high figures that were repeated were based on 939 and 1152. It can therefore be concluded that interpopulation crosses of logperch tend to be heterotic.

The survival rates for San Saba stocks are treated separately from the others in the Colorado system. The survival figures of San Saba eggs are approximately one half those of the others. Most of the difficulty occurs after hatching and apparently involves factors similar to those affecting *E. lepidum* survival.

The time lapse fertilization tests again tend to be less successful than the controls. The Brazos \times Oklahoma (high) and within-Guadalupe (low) samples are based on less than 500 eggs; those using Colorado stocks (all low) had many more than 1,000 eggs.

Hubbs (1957) reported that *Percina caprodes* sperm was more vigorous than *E. spectabile* sperm. The difference in sperm vitality is very apparent in

TABLE 5
Relative survival of interpopulation crosses of *Percina caprodes*.
Other circumstances as described in Table 1.

	Loose Cr.	Illinois R.	Oklahoma	Brazos R.	Colorado R.	Guadalupe R.
(Posthatch)						
Brazos R.			65.5		206.8	341.2
Colorado R.					188.1	197.6
Guadalupe R.	111.6	457.4				104.3
San Saba R.				434.5	0.0	214.0
(Total)						
Brazos R.			65.7		153.8	218.9
Colorado R.					124.6	106.2
Guadalupe R.	84.7	171.2				100.0
San Saba R.				109.6	103.2	101.7
(Time Lapse Fertilization)						
Brazos R.			133.7		0.0	
Colorado R.					56.4	77.2
Guadalupe R.						61.0

the experiments reported here because the sperm vitality of Central Texas logperch is at least one and one half times that of any population of *Etheostoma* analyzed in this study. The difference is probably because the logperch female produces ten to 20 eggs per spawning act, and the orangethroat produces two to seven eggs per spawning act (Winn, 1958a). Moreover, the logperch female begins spawning on the stream bottom and the orangethroat female buries herself in the gravel before spawning (Winn, 1958b). Both of these factors would result in more precise control of an individual egg's deposition site, and consequently less need for extra sperm to fertilize the scattered eggs of orangethroat darters than of logperch. The need to fertilize the "wandering" eggs would necessitate a longer sperm vitality as it is found in logperch. The reproductive pattern of the greenthroat darter resembles that of the orangethroat except that eggs are carefully applied to aquatic vegetation (Strawn, 1955). Despite the "high vigor" of logperch sperm, the duration of its vitality is relatively short with less than 1 percent fertilization after a delay of 25 seconds at 15° C.

Overall Intraspecific Survival. The interspecific hybridization experiments were mostly more successful than the controls. Only ten of 40 posthatching tests had indices below 100, and the median figure was between 116.2 and 116.6. The total hybrid survival was somewhat similar with 12 of 40 tests being below 100, and a median figure between 106.4 and 107.6. Time lapse fertilization tests were very different. Fifteen of 24 had indices below 100, and the median figure was between 73.0 and 77.2. It is readily apparent that incipient hybridization in darters is much more easily recognized with a fertilization test than by hybrid survival.

The patterns of intraspecific hybridization resemble those for interspecific hybridization listed below by being typically heterotic in survival and having a reduced fertilization rate. The heterotic responses of intraspecific hybrids had no consistent pattern with regard to distance of the separation. That is, those hybrids from the localities within the same system usually were as likely to be heterotic as those between distinct systems. The exception involves the Nueces populations of *E. lepidum* which seem to be in the process of separating from those inhabiting the Guadalupe and Colorado Systems. Except for *E. lepidum* the time lapse hybridization data also do not show geographically associated variations in fertilization potential. Sometimes the within-system hybrids are very much less successful than those between systems (Table 3), and at other times they may be relatively successful (Table 1). If this isolation mechanism were to be established in allopatry, the intensity of the mechanism should typically increase with the degree of allopatry.

There were occasional instances in which the within-system hybrids were rather unsuccessful. These may have been by chance; however, this did not occur in Colorado System tests (see below).

Intrageneric *Etheostoma* Hybrids

Comparative data on the relative survival of interspecific hybrids can provide information about the selective processes. Differences in relative survival involving direct environmental factors should have been apparent in intra-specific experiments; therefore, the repeatable patterns probably are based on genetic incompatibility.

Etheostoma lepidum × *Etheostoma spectabile*. Both reciprocals of the experiments involving these two related species were done many times. The survival of the hybrids seems to demonstrate an overall heterosis. Only two comparisons based on *E. spectabile* eggs are below 95, and excluding tests based on parents from the San Saba River, 25 are above 105 (Table 6). The

TABLE 6

Relative survival of Etheostoma spectabile ♀ × E. lepidum ♂ hybrids. A figure of one hundred (100) would be equal to that of the control experiments. Crosses within a stream system are divided into those with both parents from within five stream miles (superscript "s") and those with parents from localities separated by more than five stream miles (superscript "d").

♀ ♂	Colorado R.	Guadalupe R.	Nueces R.	San Saba R.
	(Posthatch)			
Loose Cr.	140.3	137.3		
White R.	179.9	147.2		
Illinois R.	62.1	150.0		
Brazos R.	133.8	105.3	133.6	
Colorado R.	^s 114.6 ^d 120.6	125.6	109.6	134.2
Guadalupe R.	125.8	^s 119.8 ^d 112.1	139.1	145.2
	(Total)			
Loose Cr.	107.5	104.5		
White R.	134.1	122.5		
Illinois R.	59.0	102.5		
Brazos R.	120.1	104.4	119.4	
Colorado R.	^s 102.5 ^d 100.2	105.3	100.6	102.5
Guadalupe R.	112.5	^s 105.6 ^d 104.0	115.7	123.9
	(Time Lapse Fertilization)			
Loose Cr.		137.1		
White R.	68.5		43.0	
Brazos R.	106.2	108.7	40.2	
Colorado R.	^s 65.0 ^d 40.5	87.4	75.1	
Guadalupe R.	67.2	^s 88.5 ^d 67.2	150.2	

two low tests are both Illinois \times Colorado. The low survivals are difficult to explain, but they are not repeated on the reciprocal or on other tests involving either parental population. The anomalous figures are caused by a hatching rate two thirds that of the controls. No other low survival tests have a low hatching figure and some adverse circumstances may have affected the eggs, more than 99 percent of which were collected from one locality on one day.

Ten of the reciprocal figures are below 95, and 21 are above 105 (Table 7). The somewhat reduced effect of heterosis is undoubtedly based on biologic phenomena because the low survival examples are often based on large sample sizes collected at several intervals. Two contrasting survival patterns are apparent. That using Colorado River females is invariably heterotic except when two different stocks from the same system are the parents. A similar reduction can be noted in the San Saba River tests. Comparing the success within Central Texas, the lowest survival is in experiments involving crosses between the San Saba and other Colorado stocks, or in effect, the lowest survival occurs in the within Colorado tests. Therefore, the reduced hybrid vitality occurs in experiments in which both controls had high survival rates and in those in which the controls' survival differed widely. If some extrinsic factor caused the reduced vitality of the within-system hybrids it should also

TABLE 7

*Relative survival of Etheostoma lepidum ♀ \times E. spectabile ♂ hybrids.
Other circumstances as described in Table 6.*

♀ ♂	Loose Cr.	White R.	Illinois R.	Brazos R.	Colorado R.	Guadalupe R.
(Posthatch)						
Colorado R.		162.4	135.1	120.7	^s 137.7 ^d 74.1	140.0
Guadalupe R.	75.5		65.2	84.7	124.4	^s 92.4 ^d 108.0
Nueces R.		105.9	172.2	128.3	96.7	132.2
San Saba R.		133.6	120.3	172.6	127.6	163.7
(Total)						
Colorado R.		110.6	123.2	109.2	^s 117.9 ^d 85.4	122.4
Guadalupe R.	54.1		96.6	89.4	111.1	^s 102.9 ^d 111.9
Nueces R.		87.6	109.0	111.8	84.2	111.4
San Saba R.		116.4	112.9	125.1	106.7	117.1
(Time Lapse Fertilization)						
Colorado R.		61.8	154.0	61.4	^s 128.6 ^d 105.8	371.0
Guadalupe R.				81.3	63.3	^s 142.7 ^d 51.1
Nueces R.		118.1		67.7	86.6	231.1

be apparent in the comparable intraspecific experiments which in contrast had high survival rates.

In experiments using Guadalupe system eggs there is relatively low survival, except if Colorado or other Guadalupe system males are used. These data are somewhat less reliable because the sample sizes range around 1,000, whereas the Colorado samples range about 4,000 eggs. Both the Colorado and Guadalupe comparisons show similarities between the distant and identical locality experiments, and the nearby locality tests are very different. The series are somewhat contradictory in that with Colorado system eggs the nearby survival is low and with Guadalupe eggs the nearby survival is high.

The survivals using Nueces River stocks tend to be high. Of the five combinations using *E. spectabile* males, only one has both post-hatch and total survival figures below 100 (Nueces \times Colorado). This is in striking contrast to the universally low survivals when greenthroats from different stream systems are tested.

The time lapse fertilization figures tend to be much less heterotic with only 11 figures above 105, and 17 below 95. These figures are often suspect because of small sample sizes. The crosses using *E. lepidum* eggs have seven high and seven low figures and do not show a pattern. In contrast, the reciprocals have a repeatable pattern. The one series that was studied intensively was that using Colorado River *E. lepidum* sperm. In this series only the Brazos River sample has an index above 70. Because there is no sympatric congener in the Brazos system, the eggs would not be expected to be isolated against foreign sperm. Otherwise the different Colorado sample is distinctly less successful than the others. The series based on Guadalupe *lepidum* males is nearly identical except for the successful cross with Loose Creek eggs based on a single hybrid experiment. That is, the Brazos system eggs are most easily fertilized and those from another locality within the same system the least easily fertilized. If one assumes that sympatric *E. spectabile* eggs reject foreign sperm, it should not be surprising that they also reject allopatric *E. lepidum* sperm from the Nueces River. The sample sizes for the Nueces River experiments are very low, and individual variations in the fertilization success may be due to chance.

Repeatability of the survival pattern of Colorado system hybrids in fertilization tests and the contradictions of the pattern of Guadalupe system hybrids further indicates that the former reflects a selective phenomenon.

Etheostoma caeruleum \times *Etheostoma lepidum*. Relatively few tests were made on these exclusively allopatric species. Excluding San Saba tests, six of the survival comparisons had indices over 105, and five were below 95 (Tables 8 and 9)—figures not discordant with a heterotic pattern. Moreover, the only two adequate samples present (both reciprocals of the Colorado \times White) were above 105 twice and never below 95. The tests with time lapse

TABLE 8

Survival of Etheostoma lepidium ♀ × E. caeruleum ♂ hybrids.
Other circumstances as described in Table 6.

♀ ♂	Loose Cr.	White R.
	(Posthatch)	
Colorado R.	36.9	99.2
Guadalupe R.		102.1
San Saba R.		30.2
	(Total)	
Colorado R.	66.8	112.8
Guadalupe R.		94.2
San Saba R.		69.0
	(Time Lapse Fertilization)	
Colorado R.		14.7
Guadalupe R.		30.3

TABLE 9

Survival of Etheostoma caeruleum ♀ × E. lepidum ♂ hybrids.
Other circumstances as described in Table 6.

♀ ♂	Colorado R.	Guadalupe R.	Nueces R.	San Saba R.
		(Posthatch)		
Loose Cr.	102.3	110.3		
White R.	107.9	70.4	168.4	114.3
		(Total)		
Loose Cr.	84.0	107.2		
White R.	96.0	97.4	161.2	99.8
		(Time Lapse Fertilization)		
White R.	41.5			

fertilizations are also based on small samples but all three are distinctly less successful than the controls.

Etheostoma caeruleum × *Etheostoma spectabile*. The survival of hybrids between these closely related species occupying similar ecologic niches is distinctly heterotic. Only four of the comparisons were below 95, and 29 were above 105. Survival of hybrids (Tables 10 and 11) whose parents were from two localities within the same system tends to be low. All of the indices below 100 are in this group, and none of the figures are as high as those for the same hybrid combinations taken from the same locality. Therefore, the nearby localities do far worse than either the identical or the distant parental locality hybrids.

TABLE 10
Survival of Etheostoma caeruleum ♀ × *E. spectabile* ♂ hybrids.
Other circumstances as described in Table 6.

♀ ♂	Loose Cr.		White R.	Illinois R.	Brazos R.	Colorado + Guadalupe R.
	(Posthatch)					
Loose Cr.	^s 119.8	^d 103.1	176.4	109.0		
White R.	128.1	^s 159.6	^d 96.9	141.1	125.0	145.0
	(Total)					
Loose Cr.	^s 100.9	^d 89.4	146.8	107.9		
White R.	110.2	^s 122.5	^d 78.5	115.2	112.1	98.9
	(Time Lapse Fertilization)					
Loose Cr.			285.1			
White R.		^s 144.9	^d 435.8	347.4	70.4	91.4

The time lapse fertilization experiments are somewhat contradictory. The sperm of *E. caeruleum* seems to be inhibited by *E. spectabile* eggs, but the *E. spectabile* sperm seems to be invigorated by *E. caeruleum* eggs. The inhibition is slightly greater than the apparent invigoration because the figures range between one half and one twelfth of the controls, a distinctly greater deviation than the up-to-fivefold increase, indicating an overall inhibition. There is no indication of a pattern of geographic variation except that the allopatric populations often have the least fertilization potential.

Miscellaneous Intrageneric Crosses. A variety of experiments have been carried out using two species of *Etheostoma* as parents. Most of the species combinations are based on too few populations to be useful for racial comparisons of success. The only one showing a pattern that may be meaningful is *E. punctulatum* × *E. spectabile* (Table 12). Texas *E. spectabile* seem to produce heterotic hybrids with *E. punctulatum* females; when Arkansas and Missouri orangethroat males are used there seems to be a reduced overall vitality.

The overall success of intrageneric *Etheostoma* hybrids is better than the controls. Excluding the San Saba tests, only 21 of the 118 posthatching comparisons are below 100, and the median figure is between 134.4 and 135.1; and 30 of the 118 total survival indices are below 100, and the median figure is 112.2. The time lapse fertilization experiments are distinctly different with 42 of 67 falling below 100, and the median figure is 67.2.

TABLE 11
Survival of *Etheostoma spectabile* ♀ × *E. caeruleum* ♂ hybrids.
Other circumstances as described in Table 6.

♀ ♂	Loose Cr.		White R.	
	(Posthatch)			
	^s	^d		
Loose Cr.	198.1	85.7		
White R.	134.0		^s 178.8	^d 94.6
Illinois R.				170.0
Brazos R.				139.2
Colorado R.				132.4
Guadalupe R.				130.3
	(Total)			
	^s	^d		
Loose Cr.	154.6	132.4		
White R.	118.4		^s 117.1	^d 104.0
Illinois R.				122.8
Brazos R.				117.3
Colorado R.				116.0
Guadalupe R.				115.3
	(Time Lapse Fertilization)			
Loose Cr.				40.5
White R.			^s 34.0	^d 53.5
Illinois R.				24.4
Mountain Cr.				50.9
Brazos R.				8.5
Colorado R.				15.2

Interspecific Hybrids of Primitive Darters

Only three species of primitive darters were studied extensively, *Percina caprodes*, *Hadropterus scierus*, and *H. shumardi*. Bailey, *et al.* (1954) have suggested that the species comprising the two genera are closely related and have placed all of them in *Percina*. Regardless of whether the species should be congeneric or not, there is no question that they are much more closely related among themselves than they are to any species of *Etheostoma*.

All of the species studied are difficult to rear in the laboratory. They have narrow ranges of thermal tolerance, tend to be bruised by swimming into the edges of their rearing containers, and seem to need large quantities of small food items. Consequently, survival rates vary widely, and the validity of comparisons may suffer from extrinsic factors.

Hadropterus scierus × *Percina caprodes*. The survival of F₁ hybrids is

TABLE 12

Survival of various intrageneric Etheostoma hybrids.
Other circumstances as described in Table 6.

	Posthatch	Total	Time Lapse
<i>E. punctulatum</i> ♀ × <i>E. spectabile</i> ♂			
Illinois R. × Loose Cr.	68.1	87.0	
Illinois R. × Same Illinois R.	70.9	81.8	
Illinois R. × Different Illinois R.	192.2	83.3	
Illinois R. × Colorado R.	146.0	115.4	
Illinois R. × Guadalupe R.	∞	125.2	
<i>E. spectabile</i> ♀ × <i>E. punctulatum</i> ♂			
Loose Cr. × Illinois R.	218.8	120.7	46.0
White R. × Illinois R.	215.0	154.3	139.6
Illinois R. × Same Illinois R.	239.4	129.2	40.5
Illinois R. × Different Illinois R.	160.0	124.2	283.9
Brazos R. × Illinois R.	206.2	127.0	41.5
Colorado R. × Illinois R.	194.8	132.4	
Guadalupe R. × Illinois R.	192.7	165.2	
<i>E. punctulatum</i> ♀ × <i>E. lepidum</i> ♂			
Illinois R. × Nueces R.	158.7	117.1	
<i>E. lepidum</i> ♀ × <i>E. punctulatum</i> ♂			
Colorado R. × Illinois R.	183.6	129.2	
Guadalupe R. × Illinois R.	134.4	128.4	
Nueces R. × Illinois R.	136.8	111.0	
<i>E. tetrazona</i> ♀ × <i>E. punctulatum</i> ♂			
Loose Cr. × Illinois R.	331.8	160.3	184.9
<i>E. punctulatum</i> ♀ × <i>E. tetrazona</i> ♂			
Illinois R. × Loose Cr.	235.6	67.2	
<i>E. tetrazona</i> ♀ × <i>E. caeruleum</i> ♂			
Loose Cr. × White R.	199.1	97.9	56.6
<i>E. caeruleum</i> ♀ × <i>E. tetrazona</i> ♂			
Loose Cr. × Same Loose Cr.	379.8	148.8	
White R. × Loose Cr.	485.1	164.2	
<i>E. tetrazona</i> ♀ × <i>E. spectabile</i> ♂			
Loose Cr. × Different Loose Cr.	306.2	118.8	
Loose Cr. × Same Loose Cr.	398.6	129.2	
Loose Cr. × Illinois R.	215.8	82.4	

TABLE 12 (Continued)

	Posthatch	Total	Time Lapse
<i>E. spectabile</i> ♀ × <i>E. tetrazona</i> ♂			
Loose Cr. × Same Loose Cr.	247.8	112.2	
Illinois R. × Loose Cr.	498.6	161.3	
<i>E. tetrazona</i> ♀ × <i>E. lepidum</i> ♂			
Loose Cr. × Colorado R.	187.1	79.6	
<i>E. lepidum</i> ♀ × <i>E. tetrazona</i> ♂			
Colorado R. × Loose Cr.	252.8	112.2	
<i>E. tetrazona</i> ♀ × <i>E. juliae</i> ♂			
Loose Cr. × White R.	12.0	59.8	
<i>E. juliae</i> ♀ × <i>E. tetrazona</i> ♂			
White R. × Loose Cr.	367.2	136.5	
<i>E. juliae</i> ♀ × <i>E. caeruleum</i> ♂			
White R. × Same White R.	105.0	87.2	
<i>E. caeruleum</i> ♀ × <i>E. juliae</i> ♂			
White R. × Same White R.	306.8	146.8	
White R. × Different White R.	319.9	123.7	
<i>E. juliae</i> ♀ × <i>E. spectabile</i> ♂			
White R. × Loose Cr.	77.8	97.7	
White R. × Illinois R.	228.4	153.8	
<i>E. spectabile</i> ♀ × <i>E. juliae</i> ♂			
Loose Cr. × White R.	500.1	145.2	
<i>E. juliae</i> ♀ × <i>E. lepidum</i> ♂			
White R. × Colorado R.	340.1	118.8	
White R. × Guadalupe R.	338.2	125.0	
<i>E. lepidum</i> ♀ × <i>E. juliae</i> ♂			
Guadalupe R. × White R.	398.2	125.8	
<i>E. juliae</i> ♀ × <i>E. punctulatum</i> ♂			
White R. × Illinois R.	453.0	135.5	
<i>E. blennioides</i> ♀ × <i>E. spectabile</i> ♂			
Loose Cr. × Different Loose Cr.	99.1	118.2	0
Loose Cr. × Illinois R.	90.1	105.8	0
Loose Cr. × Brazos R.	90.5	92.4	0

<i>E. spectabile</i> ♀ × <i>E. blennioides</i> ♂			
Loose Cr. × Same Loose Cr.	164.1	189.2	
<i>E. blennioides</i> ♀ × <i>E. punctulatum</i> ♂			
Loose Cr. × Illinois R.	117.6	79.0	23.9
White R. × Illinois R.			28.2
Illinois R. × Same Illinois R.			32.2
Illinois R. × Different Illinois R.			0.0
<i>E. blennioides</i> ♀ × <i>E. caeruleum</i> ♂			
Loose Cr. × White R.	107.9	80.7	174.9
<i>E. gracile</i> ♀ × <i>E. lepidum</i> ♂			
Neches R. × Colorado R.			11.1
<i>E. chlorosomum</i> ♀ × <i>E. lepidum</i> ♂			
Neches R. × Colorado R.			35.8
<i>E. chlorosomum</i> ♀ × <i>E. spectabile</i> ♂			
Neches R. × Colorado R.			28.0
<i>E. caeruleum</i> ♀ × <i>E. punctulatum</i> ♂			
Loose Cr. × Illinois R.	121.2	123.0	122.3
White R. × Illinois R.	177.3	127.7	102.7
<i>E. zonale</i> ♀ × <i>E. spectabile</i> ♂			
Loose Cr. × Illinois R.			70.5
White R. × Illinois R.			149.3
White R. × Same White R.			360.0

definitely better than that of the controls (Tables 13 and 14). Only five figures are below 95, and 22 are above 105. A pattern seems apparent in the Colorado experiments using *P. caprodes* sperm. The indices based on samples from two different localities within the system are distinctly (and significantly) lower than the others. Similar to the survival results based on *E. lepidum* and *E. spectabile* hybrids, this pattern is not apparent in Guadalupe River stocks. Unfortunately, the reciprocal experiments do not include any tests between two localities within the same system; there is, however, a distinctly lower survival of sympatric hybrids than allopatric hybrids.

The time lapse experiments are much lower than those of the controls with 13 below 95, and two above 105. It is obvious that gametic inhibition is significant in reducing natural hybridization between these two species. The patterns are less obvious, however; the lowest figures for logperch sperm

TABLE 13

Survival of Hadropterus scierus ♀ × *Percina caprodes* ♂ hybrids.
Other circumstances as described in Table 6.

♀ ♂	Illinois R.	Brazos R.	Colorado R.	Guadalupe R.
(Posthatch)				
Neches R.			167.8	314.2
Brazos R.				124.0
Colorado R.		243.2	149.5 ^s 0.0 ^d	42.8
Guadalupe R.	∞		172.7	167.4 ^s 385.4 ^d
(Total)				
Neches R.			103.8	132.1
Brazos R.				94.0
Colorado R.		147.2	113.2 ^s 64.4 ^d	66.6
Guadalupe R.	391.4		120.9	117.1 ^s 136.6 ^d
(Time Lapse Fertilization)				
Neches R.			80.8	
Brazos R.				29.1
Colorado R.		43.8	68.3 ^s 39.8 ^d	16.9
Guadalupe R.	124.5	47.9	58.4	10.1 ^s 5.1 ^d

TABLE 14

Survival of Percina caprodes ♀ × *Hadropterus scierus* ♂ hybrids.
Other circumstances as described in Table 6.

♀ ♂	Brazos R.	Colorado R.	Guadalupe R.
(Posthatch)			
Colorado R.	∞	106.6	243.9
(Total)			
Colorado R.	141.6	97.6	130.9
(Time Lapse Fertilization)			
Brazos R.	9.2		
Colorado R.		19.0	127.2
Guadalupe R.		0.0	

survival for any stream system was for those tested against female *H. scierus* from a different spot in the same stream system.

Hybridization Experiments Involving H. shumardi. The stocks used for *H. shumardi* experiments came from a single locality so the number of racial

comparisons is limited. The *H. shumardi* \times *H. scierus* survival experiments are contradictory, showing low survival with *H. shumardi* eggs and high survival with *H. shumardi* sperm (Table 15). Perhaps as a consequence no pattern can be noted.

The *H. shumardi* \times *P. caprodes* experiments are based on many more experiments and conform better to the general pattern of high survival of inter-specific hybrids. Two of the comparisons are low in both indices. The low survival of Guadalupe *H. shumardi* \times San Saba *P. caprodes* may involve the adverse environmental circumstances associated with San Saba stocks as well as the small sample available. Low survival in *P. caprodes* \times *H. shumardi* tests from the same site in the Guadalupe System seems to be real because of the large sample size. These hybrids are distinctly less viable than comparable hybrids in which the maternal parent is from a different locality.

Results of time lapse experiments based on males of either species of *Hadropterus* were high, but the number of tests was minimal because *Hadropterus* males seldom yield adequate quantities of sperm more than thirty-six hours after capture. The time lapse tests with *P. caprodes* sperm are probably more meaningful, and seem to parallel the hybrid survival tests

TABLE 15
Survival of hybrids using *H. shumardi* as a parental species.
Other circumstances as described in Table 6.

	Posthatch	Total	Time Lapse
<i>H. scierus</i> \times <i>H. shumardi</i>			
Same Guadalupe	505.2	178.5	
Different Guadalupe	378.0	176.0	194.3
<i>H. shumardi</i> \times <i>H. scierus</i>			
Guadalupe \times Neches	11.0	62.8	
Same Guadalupe	75.2	76.4	259.3
Different Guadalupe	57.6	56.0	
<i>H. shumardi</i> \times <i>P. caprodes</i>			
Guadalupe \times Colorado	240.7	111.0	181.8
Guadalupe \times San Saba	69.6	70.4	
Same Guadalupe	134.1	105.4	28.3
Different Guadalupe	204.0	93.6	9.3
<i>P. caprodes</i> \times <i>H. shumardi</i>			
Colorado \times Guadalupe	351.6	128.6	
Same Guadalupe	25.4	94.6	
Different Guadalupe	200.4	142.3	

with the best results occurring when allopatric parents are used. The absence of *H. shumardi* in all collections from the Colorado System makes the allopatric aspect of Colorado *P. caprodes* \times Guadalupe *H. shumardi* more significant.

The overall success of hybrids among primitive darters is better than that of the controls. Only seven of 21 posthatching comparisons are below 100, and the median figures are between 167.8 and 172.7. Ten of 26 total survival indices are below 100, and the median figure is between 111.0 and 113.2. The time lapse experiments are much less successful with 15 of 20 below 100, and the median figure between 29.8 and 32.8. Compared to the *Etheostoma* hybrids the effect of heterosis on F_1 survival seems to be extreme and fertilization tests show considerable inhibition. The total survival figure is intermediate, perhaps because it incorporates both F_1 survival and fertilization percentages. The apparent increase in hybrid vitality may be because the relatively low control survival rates permit greater improvement. Many *Etheostoma* controls had survivals over 70 percent so that survival of all hybrids would only result in an index of 143, a figure less than the median for primitive darters.

The exceedingly low fertilization figures may well be based on biologic criteria. The primitive darters have less control over individual egg release (Hubbs, 1955; Winn, 1958a; Hubbs and Strawn, 1957a). Therefore, the eggs might not be fertilized by sperm from the mated male and be available for fertilization by "stray" sperm. Such eggs would be expected to have a mechanism to reduce fertilization potential of foreign sperm.

Hybrids Between Primitive and Advanced Darters

Many of the experiments were carried out with hybrids between the advanced (*Etheostoma*) and primitive (*Hadropterus* and *Percina*) darters. Most species of *Etheostoma* studied (and all of these studied extensively) are relatively easily reared, and most species of primitive darters studied are difficult to rear. Therefore, the hybrid survivals are contrasted separately with the survival rates of each parent.

Percina caprodes \times *Etheostoma spectabile*. The posthatching survival of the orangethroat female \times logperch male hybrids is usually much greater than that of the controls (Table 16). It is not surprising that the hybrids always do better than the logperch controls; however, only two comparisons with orangethroats are below 95, one based on San Saba parents, and 19 are above 105. This is a very convincing example of heterosis. Both of the low comparisons with paternal orangethroats must be balanced against the comparisons with the maternal logperch survival in which the hybrid survival is more than eight and sixteen times as successful as the comparable logperch

TABLE 16

Relative survival of Etkeostoma spectabile ♀ × *Percina caprodes* ♂ hybrids. A figure of 100 outside the parentheses would mean survival equal to that of the maternal species, and inside the parentheses would mean survival equal to that of the paternal species. Crosses within a stream system are divided into those with both parents from within five stream miles (superscript "s"), and those with both parents from localities separated by more than five stream miles (superscript "d").

♂ ♀	Loose Cr.	White R.	Illinois R.	Brazos R.	Colorado R.	Guadalupe R.	San Saba R.
			(Posthatch)				
Loose Cr.	128.0 (3622)		199.3 (1139)			35.1 (854)	
White R.	209.8 (8033)						234.8 (1021)
Illinois R.		142.8 (307)	^s 101.8 (1060)	^d 121.8 (952)			127.8 (2529)
Brazos R.				112.7 (589)	125.3 (537)		122.8 (4708)
Colorado R.			122.9 No control	141.8 (1001)	^s 107.8 (414)	^d 116.4 (663)	133.6 (440)
Guadalupe R.				127.6 (555)	143.2 (594)	^s 136.0 (561)	^d 129.4 (548)
			(Total)				
Loose Cr.	94.6 (167.6)		120.0 (267.5)			70.3 (154.1)	
White R.	126.9 (275.6)						92.9 (210.2)
Illinois R.	116.0 (161.7)	102.3 (115.8)	^s 102.0 (304.8)	^d 113.4 (288.3)			95.2 (127.1)
Brazos R.				112.2 (231.1)	122.8 (177.4)	113.8 (177.4)	102.3 (188.6)
Colorado R.			116.9 No control	120.0 (254.4)	^s 97.6 (137.1)	^d 82.4 (105.6)	109.4 (168.4)
Guadalupe R.				125.0 (241.1)	136.3 (175.7)	^s 123.5 (168.1)	^d 109.1 (151.8)
			(Time Lapse Fertilization)				
White R.					70.3		
Illinois R.			^s 33.7 ^d 84.1		297.3		
Brazos R.				159.8	156.2		140.0
Colorado R.			29.5	225.0	^s 103.4 ^d 3.9	64.8	
Guadalupe R.					113.9	^s 180.6 ^d 49.6	

survival. Moreover, the Loose Creek \times Guadalupe River comparison is based on only three experiments and may not represent the actual hybrid survival. The Colorado \times San Saba comparison may reflect reduced survival of hybrids in which the parents are from two localities within the same stream system. The comparisons also may have been affected by environmental factors associated with the San Saba locality; however, the contrast with logperch is much lower than that of other hybrids whose logperch fathers came from the San Saba. Posthatching comparisons of the other interpopulation hybrids whose parents both came from within the Colorado System have a relatively high index. In contrast, overall survival figures show a low survival index for the hybrid crosses involving two Colorado System localities. The comparison with logperch is by far the lowest figure, and that with orangethroats is second to the statistically questionable Loose Creek \times Guadalupe River survival. There is also some indication of reduced vitality of Guadalupe System hybrids with parents from two localities in the system. The overall survival is somewhat less than the posthatching survival, but still compares favorably with that of the controls. All comparisons with logperch exceed 105; 14 of the comparisons with orangethroats are above 105 and five below 95.

The posthatching survivals of reciprocal experiments are also typically better than those of the controls (Table 17). All 17 comparisons with logperch are above 105 as are five of the contrasts with orangethroats. Seven of the orangethroat comparisons are below 95. The overall 23 to eight survival comparison is in accord with a heterotic pattern. On the other hand, the figures are considerably lower than those of the reciprocal. Whereas all 21 hybrid combinations with orangethroat mothers have more than four times the logperch posthatching survival rate, only six of 17 reciprocals do. Likewise, survival values of 18 of 22 comparisons of hybrids with orangethroat mothers and only five of 18 reciprocals exceed values of 105. Therefore, there is no question that a difference exists between the posthatching survival potential of the two reciprocals and that this difference parallels survival potential of the maternal parent.

The pattern of hybrid survival resembles that of *Etheostoma lepidum* females and *E. spectabile* males. The Colorado River samples from Central Texas are heterotic except for those involving two different Colorado System localities. The low figure for Colorado \times Illinois hybrids is very suspect because all of the fertilized eggs in experiments were run at 29° C., a temperature at which survivals are typically low. As in the intragenetic comparisons, the results of Guadalupe River experiments diverge with the best results obtained in crosses from nearby localities and with the worst results obtained in experiments involving sympatric or distinctly allopatric parents. The similarity in the comparison of Colorado system hybrids is striking in that both

TABLE 17

Relative survival of Percina caprodes ♀ × Etheostoma spectabile ♂ hybrids. Other circumstances as described in Table 16.

♀ ♂	Loose Cr.	White R.	Illinois R.	Brazos R.	Colorado R.	Guadalupe R.
(Posthatch)						
Loose Cr.	333 (160.6)					
White R.	261 (86.8)					
Illinois R.			548 (68.0)			
Brazos R.				1347 (141.4)	1472 (143.2)	
Colorado R.			1547 (67.6)	467 (105.9)	^s 477 (104.6)	^d 262 (69.9)
Guadalupe R.	257 (95.5)	156 (67.6)	317 (95.7)	295 (104.1)	318 (101.6)	^s 250 (88.9)
San Saba R.				1299 (60.4)	1560 (92.0)	^d 317 (89.2)
(Total)						
Loose Cr.	153.8 (120.3)					
White R.	121.4 (93.3)					
Illinois R.			174.7 (84.2)			
Brazos R.				286.3 (108.0)	344.5 (125.4)	
Colorado R.			59.7 (48.2)	163.1 (99.7)	^s 150.4 (104.0)	^d 105.3 (71.1)
Guadalupe R.	135.9 (101.8)	59.6 (43.3)	124.4 (92.2)	141.8 (103.6)	128.2 (92.0)	^s 126.6 (88.1)
San Saba R.				165.4 (70.4)	242.8 (104.0)	^d 140.3 (92.1)
(Time Lapse Fertilization)						
Loose Cr.	∞		0			
Illinois R.			359.9			
Colorado R.				44.1	^s 55.0 ^d 63.0	0
Guadalupe R.					0	^s 28.5

are based on male orangethroats and the pattern is much less apparent when female orangethroats were used.

The time lapse fertilization experiments are not heterotic, with nine comparisons above 105, and 14 below 95. The two reciprocals are quite different. The tests with *P. caprodes* sperm provide results approximately the same as the controls, especially the Texas tests in which the sample sizes are adequate. There is no question that one of the Texas comparisons is exceedingly low (different Colorado) and that it is based on a large sample. The within-Guadalupe and Illinois samples are contradictory and may not represent differential inhibition. Comparisons using *E. spectabile* sperm clearly show an overall inhibition. The two high figures are based on exceedingly small samples at time lapses in which the controls were unusually unsuccessful, resulting in an artificially high index. The adequate Texas samples are all exceedingly low, indicating that *P. caprodes* eggs have large quantities of the factor that inhibits heterospecific sperm.

Percina caprodes \times *Etheostoma lepidum*. The survival of logperch \times greenthroat darter hybrids is distinctly lower than comparable logperch \times orangethroat hybrids (Tables 18 and 19). The posthatching comparisons with greenthroats result in ten being above 105, and 24 below 95. The comparisons with logperch survivals are much higher, with all above 105. Total survival figures are comparable with the posthatching indices; eight indices above 105, and 25 below 95 when contrasted with *E. lepidum*, and 29 above 105, and two below 95 when contrasted with *P. caprodes* controls. The two reciprocals differed by having a greater survival rate when greenthroats were used as maternal parents than when logperch females were used. This is essentially the same as occurred with logperch \times orangethroat hybrids.

The comparable low survival indices of this hybrid combination as contrasted with similar indices for orangethroat \times logperch hybrids is in large part based on a low survival of sympatric crosses. The comparisons based on survival of hybrids whose logperch parent is from outside the greenthroat range indicates a reasonable degree of heterosis. Ten of the posthatching comparisons with greenthroats exceeded 105, seven of which involved allopatric logperch and in one other allopatric greenthroats were used. The other two are of dubious significance because the greenthroats used for comparison were from the San Saba River, a locality at which the control survivals were unusually (and probably artificially) low, perhaps causing a high index. The pattern can perhaps best be shown by comparing the index rankings (excluding those involving the San Saba System). The highest relative success was clearly in those crosses in which both parents were from allopatric populations (Table 20). There was no significant difference among the various comparisons within the sympatric range.

The time lapse fertilizations repeat the patterns of comparable tests involv-

TABLE 18

*Relative survival of Etheostoma lepidum ♀ × Percina caprodes ♂
hybrids. Other circumstances as in Table 16*

♀ ♂	White R	Brazos R	Colorado R.	Guadalupe R.	San Saba R.	
(Posthatch)						
Colorado R.		112.9 (628)	^s 90.2 (492)	^d 88.4 (382)	90.8 (505)	63.4 (1882)
Guadalupe R.	11.6 (6240)	77.1 (808)	78.5 (356)	^s 85.9 (383)	^d 61.8 (321)	51.4 (1350)
Nueces R.	158.6 (209)	139.6 (435)	87.8 (389)	88.4 (379)		61.3 (1204)
San Saba R.		147.0 (1440)	135.4 (388)	87.3 (243)		90.5 (1589)
(Total)						
Colorado R.		102.3 (241.4)	^s 95.0 (152.3)	^d 90.8 (151.7)	90.3 (161.2)	86.1 (151.5)
Guadalupe R.	52.3 (100.6)	85.3 (266.0)	93.1 (144.6)	^s 89.0 (151.3)	^d 81.4 (137.3)	81.8 (212.2)
Nueces R.	122.0 (122.3)	115.7 (216.7)	92.5 (138.4)	91.8 (142.2)		75.7 (152.8)
San Saba R.		120.0 (347.9)	105.0 (142.5)	73.8 (110.8)		98.1 (179.9)
(Time Lapse Fertilization)						
Colorado R.		91.8	^s 156.1	^d 134.9	162.7	
Guadalupe R.		188.5	110.5		^d 122.6	
Nueces R.		121.6	119.6		129.3	

ing orangethroat × logperch hybrids. That is, logperch sperm is not inhibited by greenthroat eggs but the reciprocal experiments show a high degree of inhibition.

Miscellaneous Intergroup Hybrids. A large number of inter-group hybrids have been reared that do not provide reliable patterns of sympatric versus allopatric survival rates (Table 21). For example, the *H. scierus* Brazos × *E. lepidum* Colorado hybrids are relatively heterotic (both reciprocals); however, both reciprocals of the equally allopatric *H. scierus* Colorado × *E. lepidum* Nueces hybrids, have low vitality and the comparable within-Colorado hybrids tend to be intermediate. Because of the small sample sizes

TABLE 19

*Relative survival of Percina caprodes ♀ × Etheostoma lepidum ♂ hybrids.
Other circumstances as in Table 16.*

♀ ♂	Colorado R.	Guadalupe R.	Nueces R.	San Saba R.
	(Posthatch)			
Loose Cr.		345 (322.2)		
White R.				135 (826.0)
Brazos R.	366 (100.4)		1440 (139.6)	
Colorado R.	^s 260 (49.8)	^d 318 (70.7)	291 (54.1)	444 (141.3)
Guadalupe R.	252 (65.1)	^s 276 (84.2)	^d 377 (66.8)	271 (90.3)
San Saba R.	1667 (49.2)			1495 (71.9)
	(Total)			
Loose Cr.		175.3 (205.1)		
White R.				91.3 (137.8)
Brazos R.	261.2 (107.0)		320.2 (112.5)	
Colorado R.	^s 96.0 (64.4)	^d 120.2 (74.4)	94.9 (59.9)	130.3 (91.2)
Guadalupe R.	114.2 (66.5)	^s 132.0 (80.3)	^d 99.7 (77.0)	116.4 (85.2)
San Saba R.	159.2 (60.4)			200.3 (75.4)
	(Time Lapse Fertilization)			
Brazos R.	45.1			
Colorado R.	^s 37.2	^d 21.1	5.3	
Guadalupe R.	25.5	^s 19.9	^d 42.4	14.0

available, those variations make no sense and may be influenced by extrinsic factors.

Several items are available that substantiate the circumstances present in other intergroup hybrids. The two reciprocals differ in survival potential in

TABLE 20

Average rankings of survival indices of
greenthroat \times logperch hybrids

	<i>P. caprodes</i> eggs	<i>E. lepidum</i> eggs	Total
Both parents allopatric	1.8	5.0	6.8
<i>P. caprodes</i> allopatric	2.9	6.3	9.2
<i>E. lepidum</i> allopatric	4.8	7.9	12.7
Different sympatric systems	9.5	6.9	16.4
Different sympatric localities	6.8	9.5	16.3
Same localities	8.1	6.6	14.7

TABLE 21

Survival of various intergroup darter hybrids.
Other circumstances as in Table 16.

	Posthatch	Total	Time Lapse Fertilization
<i>(H. scierus</i> ♀ \times <i>E. lepidum</i> ♂)			
Neches R. \times Guadalupe R.			0.0
Neches R. \times Nueces R.			0.0
Brazos R. \times Colorado R.	165.4 (112.4)	128.0 (102.8)	12.8
Colorado R. \times Same Colorado R.	196.9 (58.0)	143.3 (76.4)	31.2
Colorado R. \times San Saba R.	617.9 (115.0)	300.8 (107.1)	57.2
Colorado R. \times Nueces R.	76.6 (70.6)	62.0 (57.9)	10.7
Guadalupe R. \times Colorado R.	425.9 (87.2)	177.0 (92.1)	4.9
Guadalupe R. \times Different Guadalupe R.	272.4 (25.6)	101.8 (44.6)	
Guadalupe R. \times Nueces R.	678.9 (67.6)	152.5 (68.1)	

<i>(E. lepidum</i> ♀ \times <i>H. scierus</i> ♂)			
Colorado R. \times Brazos R.	134.2 (∞)	120.6 (302.4)	692.4
Colorado R. \times Same Colorado R.	79.2 (245.4)	89.5 (159.9)	94.9
Colorado R. \times Guadalupe R.	101.4 (303.9)	103.6 (158.2)	
Guadalupe R. \times Neches R.	0 (0)	3.6 (7.4)	970.6
Guadalupe R. \times Brazos R.	96.9 (181.7)	106.7 (143.5)	

TABLE 21 (Continued)

	Posthatch	Total	Time Lapse Fertilization
Guadalupe R. × Colorado R.	126.3 (378.9)	117.9 (240.8)	2428.6
Guadalupe R. × Different Guadalupe R.	145.4 (400.6)	117.6 (176.1)	
Nueces R. × Neches R.	54.2 (284.1)	70.0 (128.6)	481.2
Nueces R. × Colorado R.	86.8 (278.6)	88.7 (137.0)	22.3
Nueces R. × Guadalupe R.	116.0 (294.1)	95.2 (136.0)	
San Saba R. × Brazos R.	137.2 (121.1)	120.6 (117.9)	
San Saba R. × Colorado R.	121.3 (149.9)	106.8 (133.7)	
San Saba R. × Guadalupe R.	160.3 (376.1)	118.4 (166.6)	
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<i>(H. scierus ♀ × E. caeruleum ♂)</i>			
Colorado R. × White R.	87.3 (52.8)	87.3 (34.0)	
Guadalupe R. × White R.	208.8 (58.2)	61.0 (50.9)	
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<i>(H. scierus ♀ × E. spectabile ♂)</i>			
Neches R. × Colorado R.	187.0 (78.4)	102.8 (69.6)	18.7
Colorado R. × White R.	109.5 (67.4)	96.2 (65.0)	2.7
Colorado R. × Brazos R.	197.6 (90.0)	126.1 (83.5)	53.4
Colorado R. × Same Colorado R.	313.6 (113.3)	170.1 (102.4)	81.8
Colorado R. × Different Colorado R.	661.1 (69.0)	219.9 (109.5)	31.7
Guadalupe R. × White R.	242.2 (75.4)	121.7 (68.4)	53.7
Guadalupe R. × Illinois R.	51.0 (9.5)	94.1 (54.8)	8.3
Guadalupe R. × Brazos R.	347.9 (72.8)	167.2 (89.2)	22.9
Guadalupe R. × Colorado R.	557.1 (131.8)	213.1 (110.0)	47.5
Guadalupe R. × Same Guadalupe R.	473.2 (149.2)	167.2 (117.0)	24.5

Guadalupe R. × Different Guadalupe R.	311.9 (87.8)	153.8 (92.9)	
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<i>(E. spectabile ♀ × H. scierus ♂)</i>			
White R. × Colorado R.	125.0 (92.1)	107.3 (103.2)	
Brazos R. × Colorado R.	98.9 (269.2)	106.3 (176.0)	264.1
Brazos R. × Guadalupe R.	103.2 (343.2)	103.9 (159.8)	240.0
Colorado R. × Neches R.	162.0 (307.4)	134.8 (165.3)	151.1
Colorado R. × Same Colorado R.	125.4 (271.4)	104.1 (151.3)	68.9
Colorado R. × Guadalupe R.	85.6 (304.0)	82.6 (146.9)	25.6
Guadalupe R. × Colorado R.	265.6 (302.1)	189.0 (176.9)	
Guadalupe R. × Same Guadalupe R.	130.4 (446.9)	139.4 (190.9)	236.2
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<i>(H. shumardi ♀ × E. spectabile ♂)</i>			
Guadalupe R. × Brazos R.	338.0 (67.0)	156.8 (79.1)	
Guadalupe R. × Different Guadalupe R.	449.2 (118.2)	187.4 (111.7)	44.6
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<i>(E. spectabile ♀ × H. shumardi ♂)</i>			
White R. × Guadalupe R.			0
Brazos R. × Guadalupe R.	160.8 (454.7)	145.4 (258.0)	
Colorado R. × Guadalupe R.	129.0 (744.3)	119.8 (249.6)	0
Guadalupe R. × Different Guadalupe R.	108.2 (827.8)	106.2 (229.3)	0
<hr/>			
<i>(H. shumardi ♀ × E. lepidum ♂)</i>			
Guadalupe R. × Colorado R.	281.9 (48.8)	164.1 (67.4)	
Guadalupe R. × Different Guadalupe R.	70.2 (9.8)	108.0 (44.1)	
<hr/>			
<i>(E. lepidum ♀ × H. shumardi ♂)</i>			
Colorado R. × Guadalupe R.	95.4 (376)	96.0 (185.0)	
Nueces R. × Guadalupe R.	133.7 (1135)	118.7 (268.9)	

TABLE 21 (Continued)

	Posthatch	Total	Time Lapse Fertilization
<i>(E. caeruleum</i> ♀ × <i>Percina caprodes</i> ♂)			
Loose Cr. × Loose Cr.	133.4 (818)	116.9 (211.4)	∞
Loose Cr. × White R.	133.3 (195)	125.2 (122.3)	
Loose Cr. × Illinois R.	132.9	125.8	
	No Control	No Control	
Loose Cr. × Guadalupe R.	115.0 (343)	108.8 (188.6)	113.6
White R. × Loose Cr.	133.0 (2689)	108.8 (212.1)	100.0
White R. × White R.	387.1 (39043)	155.7 (234.5)	
White R. × Illinois R.	130.4	117.9	77.9
	No Control	No Control	
White R. × Colorado R. + Guadalupe R.	148.2 (433)	117.2 (162.4)	110.4
White R. × San Saba R.	124.8 (1864)	101.9 (239.2)	
<i>(Percina caprodes</i> ♀ × <i>Etheostoma caeruleum</i> ♂)			
White R. × White R.	190.0 (115.0)	137.2 (124.8)	29.4
Guadalupe R. × White R.	200.5 (49.8)	91.3 (60.4)	40.2
<i>(E. tetrazona</i> ♀ × <i>Percina caprodes</i> ♂)			
Loose Cr. × Loose Cr.	55.3 (232.1)	117.0 (167.8)	
Loose Cr. × White R.	175.8 (316.4)	174.8 (162.5)	
<i>(Percina caprodes</i> , ♀ × <i>Etheostoma punctulatum</i> ♂)			
Colorado R. × Illinois R.	345.0 (180.6)	150.3 (139.3)	15.3
Guadalupe R. × Illinois R.	303.4 (180.0)	106.9 (91.0)	63.8

that the hybrids based on *Etheostoma* eggs are more viable than those based on *Percina* or *Hadropterus* eggs. Similarly, both reciprocals of hybrids with *E. spectabile* tend to be more successful than those based on *E. lepidum*. Finally, *Percina* and *Hadropterus* eggs tend to inhibit *Etheostoma* sperm, whereas the reverse is less apparent.

Overall Intergroup Hybridization. The relative survival of intergroup

hybrids follows the same pattern regardless of species involved (Table 22). The reciprocal based on *Etheostoma* eggs does better than that based on primitive darter eggs. The median survival figure is invariably higher, when either *E. lepidum* or *E. spectabile* are crossed with primitive darters. Comparisons using only *P. caprodes* eggs follow the same pattern but include a slightly higher figure in one comparison with *E. lepidum*. Using all primitive darters, three of the differences between reciprocals are significantly more successful with *Etheostoma* eggs than with primitive darter eggs at the 0.05, 0.01, and 0.0001 levels.

Superimposed on this pattern is a difference in the success of *E. lepidum* and *E. spectabile* when they are crossed with primitive darters. All 16 possible ways of comparing the survival medians result in higher survival when *E. spectabile* is used as the specialized darter for hybridizations. The 16-0 comparison is slightly exaggerated because many of the tests are in effect using the same data. One of the comparisons is significant in itself at a level between 0.01 and 0.001. It is quite apparent that the *E. spectabile* \times primitive darter are much more likely to survive than are *E. lepidum* \times primitive darter hybrids.

The results with time lapse fertilization tests are somewhat similar in that primitive darter eggs significantly inhibit *Etheostoma* sperm, and primitive darter sperm does not seem to be adversely affected by *Etheostoma* eggs.

TABLE 22

Median index of success of various intergroup hybrids. The indices are obtained from Tables 16-21. Those limited to P. caprodes as the primitive parent are shown in parentheses. If even numbers of indices are available, the two medians were averaged.

	<i>spectabile</i> ♀	Primitive ♀	<i>lepidum</i> ♀	Primitive ♀
Posthatch <i>Etheostoma</i> comparison	127.6 (127.7)	90.0 (98.6)	90.5 (88.1)	71.3 (90.3)
Posthatch Primitive comparison	561 (575)	333 (400)	382 (464)	287 (318)
Total <i>Etheostoma</i> comparison	109.4 (110.8)	92.6 (93.3)	95.0 (91.3)	75.9 (77.0)
Total Primitive comparison	176.4 (177.4)	146.1 (141.3)	151.7 (151.6)	129.2 (120.2)
Time lapse reduction in sperm activity	93.9 (103.4)	38.2 (44.1)	132.1 (126.0)	19.9 (23.3)

This circumstance cannot account for the differential results with hybrid survival because that pattern is very apparent in posthatching comparisons.

The differences between the results using the two specialized darters is not retained in the fertilization tests. One comparison favors *E. spectabile* and the other (incidentally that one that is statistically significant), favors *E. lepidum*. Obviously, the factors that caused the survival of intergroup *E. lepidum* hybrids to be lower than that of comparable *E. spectabile* hybrids do not affect fertilization potential, and it seems likely that the circumstances are reversed.

The overall hybrid survivals are less easily determined because the two controls are so different that comparisons with both parents together is impossible. The values listed below are those in which all figures are used and then each parent is contrasted separately. Seventy of 280 posthatching comparisons are below 100 (65 of 143 *Etheostoma* and 5 of 137 primitive), and the median figure is 161 (104 in *Etheostoma*, and 379 for primitive controls). Eighty-two of the 280 total comparisons are below 100 (70 of 143 *Etheostoma*, and 12 of 137 primitive darter controls), and the median figure is 118 (102 *Etheostoma*, and 154 for primitive controls). The figures for time lapse fertilization are much lower with 52 of 83 below 100 (36 of 38 for *Etheostoma*, and 16 of 44 for primitive darters); the median figure is 57 (28 for *Etheostoma* and 120 for primitive darters).

Discussion

A variety of conclusions are available concerning geographic variation of adaptive characters, and those emphasized in this discussion relate to the action of one species on another. The best approach to this problem is to use a wide-ranging species. Those populations occupying areas outside the range of most other species would be subject to different competitive factors than would those populations sympatric with a number of near relatives. Those species with narrow geographic limits could of course be modified by selection influenced by the presence of relatives; but the effects would be difficult to ascertain because of the absence of an unaffected homospecific population.

Egg Size. The egg size of a darter is roughly inversely proportional to the egg number. The abdominal cavity of a female can contain a given volume of eggs. Any increase in egg number would consequently result in an equivalent decrease in egg size (See Hubbs and Johnson, 1961, for measurements of darter eggs associated with egg complements). Longer females have considerably larger abdominal cavities and consequently a higher fecundity. The increase in egg number with size should optimally approximate the cube of the length increase. When food is in short supply the increase is less because food intake would limit the nutrients available for egg production. The largest

females often have very few eggs, probably because food intake must increase more or less in a linear fashion, and metabolic needs increase logarithmically (See Hubbs, 1964b, for a discussion of some aspects of this problem). Because of these non-genetic influences, egg size is a more meaningful index of fecundity than egg numbers obtained at a single observation interval. No seasonal or significant growth changes in egg size have been noted in the darters studied.

The space available for mature eggs also would be affected by requirements for other abdominal structures. Overlooking this factor may have led Williams (1959), who used ovary weight as an index of fecundity, to draw an erroneous conclusion. Darters with extensive parental care had proportionally heavier ovaries than did those with little parental care. Superficially this indicates a greater fecundity with increased parental care. On the other hand, if parental care is extensive, much postspawning effort would be devoted to the care of the eggs, delaying the next spawning interval and reducing the need to maintain a series of nearly ripe eggs in the ovary. Overcrowding the ovary might crush the developing eggs in "non-care" darter females. As a result there would be selective pressure to maintain a relatively small ovary. Therefore, Williams' (1959) data can be interpreted to show that parental care is associated with low fecundity. Actually, fecundity involves the number of mature eggs produced optimally in a reproductive season. Those darters with many spawning intervals (no parental care) would be much more likely to produce many more eggs than those with few spawning periods (much parental care).

Egg sizes have been ascertained for a number of populations of darters. The two most extensively and intensively studies were *E. spectabile* and *P. caprodes*. Both have small eggs in Missouri, intermediately sized eggs in Arkansas, and big eggs in Texas. Similarly, within Texas the western populations have bigger eggs than eastern populations at the same latitude. The intraspecific association of small northern or eastern eggs is widespread; *E. zonale*, *E. blennioides*, and *E. caeruleum* from Missouri all have eggs at least one-tenth of a millimeter less in diameter than those from Arkansas. The smaller eastern and northern *H. scierus* and *E. lepidum* eggs can reflect either of the two clines. These data indicate increased fecundity in eastern and northern darters. The latter is in distinct contrast with the generalization by Hesse *et al.* (1937, pp. 159 and 160), and Moore (1942) that large eggs are associated with low temperatures. The pattern of increased fecundity with northern samples is similar to that obtained by Tinkle (1961) for a turtle. It is possible that the apparent discordance does not exist but merely reflects the interpretation of the phenomena. Most of the literature cited by Hesse *et al.* (1937) concerns cold temperature animals. Both Tinkle's and the present studies include a large warm temperate element. A similar parallel can

be drawn with Moore's *Rana* work. His early work (1942) showed large eggs associated with cold (northern) waters; however, later (1949) he obtained large eggs in Mexico. Addition of warm temperate samples again resulted in large southern eggs. The available comparisons indicate that the north-south cline in darter fecundity is not as striking north of Missouri. Comparisons with Winn's (1958a) egg size data from Michigan show that while *P. caprodes* and *E. spectabile* have slightly smaller eggs than those recorded here from Missouri, the Michigan measurements for *E. blennioides*, *E. caeruleum*, and *E. flabellare* show that these species have bigger eggs than in Missouri. Likewise, the last two also have bigger eggs in New York, a west-east increase in egg size. Both clines seem to be reversed. It is possible that reversal of the clines is associated with a single selective factor. Missouri is near the center of distribution of darters. Far more species are available within one general area than would occur in Michigan, New York, or Texas. Similarly, more species are found in East Texas than West Texas, and in southern Michigan than in New York. Therefore, large eggs and low fecundity are associated with few species of darters, and small eggs and high fecundity occur together with many species. It is obvious that intraspecific competition is usually more severe than interspecific competition. This seems likely in darters, especially in the postlarval stages when some occupy midwater and the others are on the bottom. All adult darters are bottom fishes. Therefore, more habitats are available to larval darters than to their parents so that two sympatric species are more likely to compete as adults than as larvae. An area with few species would have more larval competition than a similar area with many species. Large eggs would permit greater individual survival through intensively competitive larval stages because of ample yolk supplies. Small eggs would permit more larvae and if larval competition were reduced, more subadults when that species entered the competitive environment. This is essentially the conclusion made by Hubbs and Delco (1960) for fecundity variations in *Etheostoma lepidum*, and is extended here to the majority of the darter egg size geographic variations. The weakest aspect in the difference in fecundity of darters is north and east of Missouri. This part of the U-shaped pattern of darter egg sizes happens to resemble that reported by Hesse *et al.* (1937). Not only are the darter estimates based on small sized samples, they are also subject to potential differences in measuring techniques. For instance, Fahy (1954) measured *E. blennioides* eggs that averaged larger than any others. His measurements were obtained from eggs extracted from females preserved in alcohol. These eggs, if laid, would have been much larger than Fahy's measurements indicated, because they had not had an opportunity to swell in natural waters and in fact had been subject to alcohol shrinkage. The competition-based cause for egg size variation may also be applied to Moore's (1949) *Rana* data. The smallest eggs come from Florida

where many anuran species are found; the largest occur in Quebec where there are few anurans, and in Mexico where there are only two species of ranids.

The differences in egg size between the Illinois and White systems may be somewhat discordant to the general pattern listed above. Three of four species with different sized eggs, *P. caprodes*, *E. stigmaeum* and *E. spectabile*, have larger eggs in the White, and only *E. blennioides* has large eggs in the Illinois. The White collections are on approximately the same latitude as those from the Illinois, but the White River drains toward the north and has a more northern fauna. On the other hand, both have a variety of species that is approximately equal to the other. The details of the species distributions may show that the competitive conditions are severe in the White.

It is apparent that interspecific fecundity variations do not follow a consistent north-south pattern. There is some evidence, however, that the interspecific variations may show fewer larger eggs in the north. Interspecific variations are of course subject to many selective factors. For instance, Winn (1958a) stated that *E. flabellare* had the most elaborate parental care of any darter. This species also has the largest eggs of any darter measured by Winn, Lake (1936), and me. Therefore, large eggs and low fecundity are associated with parental care. Because parental care tends to be equivalent within taxa, the pattern within species groups may be the best index of geographic effects on species. Wherever they are sympatric, *E. caeruleum* has larger eggs than *E. spectabile*, and the former is of more northern distribution than the latter. Similarly, the northern *E. nigrum* has bigger eggs (1.5 millimeters, Winn, 1958a; and Speare, 1965) than the southern *E. stigmaeum* (1.1–1.4 millimeters), and *E. chlorosomum* (1.1 millimeters).

Hybrid Fertility. Although any darter hybrid combination can be reared to mature size as easily as the controls, this does not demonstrate that genetic material can be exchanged. The minimal data available indicate that hybrid fertility is associated with morphologic similarity, and in effect reflects phylogenetic divergence. *Etheostoma grahami* and *E. lepidum* are closely related allopatric species that have fertile hybrids. Both species are reasonably closely related to *E. spectabile* and the hybrid females are fertile, the males sterile. Similarly, *E. radiosum* is reasonably closely related to *E. spectabile* and these hybrids are also fertile if female and sterile if male. A few hybrids have been reared between one or more of the above species and several less closely related species of *Etheostoma*; and no viable gametes in the sexual dimorphic adult-sized hybrids were noted. Similarly, in the putative hybrids between *Percina caprodes* and *Hadropterus scierus* both sexes seem to be sterile (Hubbs and Laritz, 1961b). All hybrids reared from parents belonging to the two groups of darters show no signs of sexual dimorphism. Examination of the gonads shows that they are reduced or absent. Hybrid fertility varies ex-

tensively and the degree of fertility is associated with the phylogenetic relationship of the parents.

Patterns of Hybrid Survival—Phylogenetic Differentiation. The potential of producing viable hybrid adults seems to be absolute. It is likely that any darter hybrid combination can be raised to adult size (Hubbs and Strawn, 1957a; and Hubbs, 1959) and that darters crossed with members of other taxa cannot be reared past prolarval stages (Hubbs, 1967). The only potential intermediate stage might occur in etheostomatine \times percine hybrids, a combination not yet tested. The definitive hybrid success seems to be typical of many teleosts (Hubbs, 1955) and distinctly different from the circumstances in other vertebrates such as *Bufo* (Blair, 1961) or many insects such as *Drosophila* (Patterson and Stone, 1952). Cyprinodont fishes are the only North American ones clearly shown to have intermediate levels of hybridization potential (Hubbs and Drewry, 1960); however, reduced survival of distant centrarchid hybrids (West and Hester, 1966) and deformation of salmonid hybrids (Crossman and Buss, 1966) indicates that intermediate survival rates may be widespread. The cobitid fishes of Asia also seem to have reduced survival of some hybrid combinations (Suzuki, 1957, etc.)

Comparison of survival invariably results in relatively greater success in hybrids (Table 23). This heterotic survival parallels typically increased growth rate of hybrids (Ricker, 1948; Smirnov, 1953; and Guidice, 1966). The survival of individual crosses varies widely due to chance circumstances; however, the crosses done extensively are typically heterotic as are the majority of those seldom done. The percentage above the control survival rate and the median indices would show the general effect of phylogenetic differentiation. It is apparent that the hybrid survivals are better than those of the controls. More critically, the intergeneric hybrids are at least as successful as the controls. For the most part they are intermediate between the survival of the interspecific hybrids within either group. The high incidence of heterosis is especially significant when one considers that hybrids of species not yet reared were excluded from the various hybrid survival rates discussed. Obviously, inclusion of those successful combinations, regardless of the level of success, would raise the various indices. Therefore, phylogenetically the hybrids are either very successful or, if between families, unable to be reared.

The high survival rates for intergeneric hybrids clearly shows that the capacity to produce hybrids has no direct application to conspecific relationship of the parental taxa. It merely signifies that the parental taxa are relatively closely related.

Patterns of Hybrid Survival—Differences between reciprocals. Many hybrids have been shown to have differential survival of the reciprocals (Patterson and Stone, 1952; Thornton, 1955; Hubbs and Drewry, 1960; and many others). One reciprocal is viable (and often heterotic) and the other inviable.

None of the darter hybridization tests provide such absolute differentiation of hybrid survival. Hybrids between primitive and advanced darters do vary quantitatively in survival, depending upon which reciprocal is used. Such survival variations are less easily recognized than the absolute ones typically recorded in the literature. The intergroup hybrids based on primitive darter eggs are less viable than are the intergroup hybrids based on specialized darter eggs. The difference between reciprocals parallels difference between rates of the maternal parents.

These reciprocal differences indicate considerable maternal influence on the hybrid. It is likely that maternal influence also may apply to morphological characters because both reciprocals of atherinid hybrids are more like their maternal than their paternal parents (Rubinoff and Shaw, 1960).

Differences between reciprocals is somewhat discordant with Nikoljugin's (1952) hypothesis of hybridization indicating the primitive parent. He theorized that F_1 hybrids should more closely resemble primitive parents than specialized parents. Reciprocal differences clearly show that both reciprocals should be reared in order to determine primitive parents, because one reciprocal might resemble its mother. Similarly, many F_1 hybrids show fantastic morphological variation (Hubbs, 1956; Hubbs and Strawn, 1957c; Suzuki, 1957; etc.) so that small samples might not be at all meaningful. All of these problems could distort data so that Nikoljugin's hypothesis should be applied only with extreme caution.

The overall survival data for intergroup experiments are more discordant with Nikoljugin's hypothesis. Each hybrid reciprocal of the *E. spectabile* \times *P. caprodes* hybrids does as well as or better than both controls. Because *E. spectabile* always has a higher survival potential than *P. caprodes*, the hybrid survival is invariably more like *E. spectabile* than like that of *P. caprodes*. Following Nikoljugin, the specialized darters would then be primitive and the primitive darters specialized, a conclusion discordant with all morphologic evidence.

Patterns of Hybrid Survival—Ecologic vs. Geographic Factors. The relative significance of various factors in speciation has been discussed extensively by many authors. A number of workers, notably Mayr (1963), have held that species separation nearly always involves geographic isolation, and others such as C. L. Hubbs (1961) have held that ecologic selection can be sufficiently strong to permit species to separate in sympatry. Analysis of the relative survival of hybrids may apply to this discussion. The two most intensively studied species of *Etheostoma* - *lepidum* and *spectabile* have distinctly different ecologic niches as larvae. *E. spectabile* lives on the bottom and larvae of *E. lepidum* are free swimming. The larvae of all species of primitive darters are also free swimming. Therefore, during the larval stages studied, *E. lepidum* was competing with primitive darters and *E. spectabile* was not. In

contrast, the geographic relationship is reversed. That is, *E. lepidum* frequently is allopatric to primitive darters and *E. spectabile* seldom, if ever, is allopatric. Contrasted with their controls hybrids between primitive darters and *E. lepidum* are distinctly less viable than those between primitive darters and *E. spectabile* (Table 22). The reduction in hybrid vitality of ecologically overlapping forms compared with geographically overlapping taxa indicates ecologic factors have a greater effectiveness than geographic factors.

The time lapse data appears to contradict the relative effectiveness of geographic and ecologic selection because *E. lepidum* eggs inhibit primitive sperm significantly less than do *E. spectabile* eggs. The contradiction is apparently not real because the ecologic circumstances are reversed during egg deposition. That is, the primitive darters studied here and *E. spectabile* lay eggs in or on gravel and *E. lepidum* attaches its eggs to aquatic vegetation. Therefore, this comparison also substantiates the significance of ecologic factors in developing isolation mechanisms.

The above evidence supports ecologic isolation as being highly significant in speciation. Livingstone (1965) also has emphasized the role of ecologic factors as isolating mechanisms by showing that geographic isolation in African lakes could not have occurred as hypothesized by Brooks (1950).

Patterns of Hybrid Survival—Gametic Inhibition. The indices for time lapse fertilization (Table 23) are distinctly lower than those of the controls. This reduction in fertilization potential clearly involves the time lapse phenomenon, because a similar analysis of fertilization indices obtained from "damp pan" experiments result in 48 percent of the indices below 100, and a median index of 97. The results do not deviate significantly from 50 percent and a median index of 100, but do deviate significantly from all of the time lapse sum-

TABLE 23
Comparison of hybridization success with level of phylogenetic differentiation.

	Interpopulation Interspecific	Interspecific Specialized	Interspecific Primitive	Between Groups
% combinations reared	56 (all controls)	69		62
% posthatching indices above 100	75	82	67	75
Median index	116	135	170	161
% total indices above 100	70	75	62	71
Median index	107	112	112	118
% time lapse indices above 100	38	37	25	37
Median index	75	67	37	57

maries presented on Table 23. The relative significance of "sperm delay" in the gametic inhibition of darters is also shown by the equivalent results of experiments with up to ten seconds delay and the strikingly divergent results of experiments with greater delay. The slightly (and insignificantly) reduced fertilization percentages of "damp pan" experiments may reflect a time lapse related circumstance. Some of the eggs would have been located distant from any of the sperm and the two gametes might have come in contact after considerable delay. The fertilization of those eggs would therefore reflect inhibition of "tired" sperm by heterospecific eggs.

The great difference in the relative fertilization potential between fresh and exposed sperm shows that this isolating mechanism would have little effect on behavioral accidents. That is, those heterospecific pairs that mismated would not have a significantly reduced rate of fertilization (if the summarized data are correct approximately 97 percent of the homospecific rate). In contrast, those sperm that are washed away from the vicinity of the mated pair might be swept by the current to the vicinity of a second mated pair. If this pair were of the same species the chances of the "tired" sperm fertilizing an egg would be considerably less than the fresh sperm, and the relative success would depend upon the time of the exposure and temperature of the water. If, on the other hand, the second mated pair were of a different species, the phenomena responsible for the time lapse results would also apply to the fertilization potential. At best, the probability of heterospecific fertilization would be 67 percent of that of comparable homospecific pairs from the same locality. Therefore, this isolation mechanism is useful in preventing the chance meeting of gametes, and shows the strong selection in darters against hybridization. This factor is further shown by the exceedingly short duration of sperm activity—up to 25 seconds at 15° C. This figure is notably shorter than all listed by Lindroth (1947) for Swedish fresh-water fishes, by Fink and Hayden (1960) for marine cottoids, by Miller (1952) for a marine clupeid, and by Hubbs and Drewry (1958) for euryhaline cyprinodonts. Darters breed in rapids, a factor that otherwise would increase potential chance hybridization. The very low duration of sperm vitality would be required to prevent massive hybridization in oviparous animals breeding in rapid water. The short duration of brown trout sperm fertilization potential (about two minutes at 9° C.) (Buss and Carl, 1966) probably reflects their rapid water spawning site. The exceedingly low incidence of natural darter hybrids indicates the effectiveness of this isolation mechanism. Only the intergroup hybrid reported by Hubbs and Laritz (1961a) clearly resulted from chance gametic contact.

There is considerable difference in the effects of primitive darter eggs and those of specialized darters on sperm vitality. The difference is apparent from the data presented in Table 23 for within-group hybrids (median indices of

37 and 67 respectively) and equally divergent in the between-group hybrids listed on p. 57 (median indices of 28 and 120 respectively). Therefore, primitive darter eggs have a much greater inhibition of foreign sperm than do those of specialized darters. Primitive darter females produce many eggs at spawning time and typically lay their eggs on the surface of the substrate. Specialized darters produce fewer eggs and have greater control over deposition of eggs which are typically placed under gravel, on the underside of rocks, or on aquatic vegetation. Scattered eggs would be most exposed to chance fertilization and these have the greatest inhibition against sperm which might be carried to them by currents.

Gametic inhibition is somewhat intermediate between premating and postmating isolating mechanisms. It does inhibit fertilization and therefore could be considered to be premating. The egg that was exposed to foreign sperm would be likely to have been washed away from the mated pair before a homospecific sperm joined it. Therefore, this type of egg would be unlikely to produce a homospecific embryo and would be already wasted. If so, the population model for selection discussed below would apply. In contrast, any egg that was contacted by the foreign sperm while still in the swarm of homospecific sperm and before it had been fertilized by homospecific sperm could, of course, produce a homospecific embryo from a subsequent sperm contact and be subject to individual selection if a means of avoiding heterospecific fertilization were present.

Patterns of Hybrid Survival—Reinforcement. Only one series of experiments (with logperch and greenthroat darters) provided distinct evidence for reinforcement. Both reciprocals of this hybrid combination had distinctly higher survival potential if both parents were from allopatric stocks; intermediate survival potential if only one parent was allopatric; and low survival potential if both parents were from sympatric stocks (Tables 18–20). The relative difference is indicated by the fact that hybrids with both parents from allopatric stocks never had indices below 100 (median 150), those with one allopatric parent were below one hundred, 29 percent of the time (median 138), and those with both parents sympatric had indices below one hundred 49 percent of the time (median 101). Except for the most extreme figures, the percentage above a given figure is always highest in allopatric crosses and always lowest in sympatric crosses, and in most instances with a probability of less than 0.01 of being by chance. The exceptional circumstances never approach statistical significance; therefore, the probability of allopatric populations producing viable hybrids is approximately 1½ times that of comparable sympatric populations assuming the geographic isolation is eliminated.

A similar reinforcement of isolation mechanisms is indicated by the *H. shumardi* × *P. caprodes* hybrids and for *E. punctulatum* female × *E. spectabile* male crosses. The only possible reduced hybrid vitality in allopatry is

in the *E. tetrazona* \times *E. spectabile* hybrids; however, too few eggs from too few localities were used to draw even tentative conclusions. Low survival of greenthroat \times rainbow darter hybrids may indicate low hybrid potential in allopatric crosses but these data are contradicted by the highly successful hybrids of *E. lepidum* and *E. juliae* which are equally allopatric.

Reinforcement of isolation mechanisms also is supported by the exceedingly high vigor of *E. spectabile* sperm in control experiments from the Brazos system, an area in which no other species of *Etheostoma* have been collected. This seems also to be reflected in the nil effect of Brazos *E. spectabile* eggs on *E. lepidum* sperm from sympatric areas. The low vigor of equally allopatric *E. lepidum* sperm may be rationally explained by the small quantities necessary for fertilizing the reduced egg complements there. The Brazos *E. spectabile* egg complements are nearly equivalent to those of the Colorado and Guadalupe systems.

The logperch \times greenthroat darter combination clearly involves greater hybridization potential in allopatry than in sympatry. It follows the theoretical model set up by many authors for reinforcing isolation mechanisms. It is, however, the first example of reinforcement applicable to postmating isolating mechanisms. The standard explanation of reinforcement based on premating mechanisms—those individuals that do not waste gametes in producing hybrids have more gametes available for homospecific zygotes—cannot apply here. Any zygote whose survival potential is reduced must already have resulted in wasted gametes.

Because individual selection is not readily applied, population level selection may be utilized. Most darters occupy similar habitats and will occupy adjacent habitats if the usual occupant is absent. For example, *E. spectabile* is found in gravel riffles and *E. lepidum* in riffles with abundant aquatic vegetation when sympatric. In contrast, each will occupy both habitats when the other is absent. It is likely that hybrid darters occupy habitats similar to those of their parents. If these darters are heterotic they might easily displace one or both parental species in their preferred habitat. For example, large numbers of putative hybrids between *E. lepidum* and *E. spectabile* have been collected in the Guadalupe River near Kerrville State Park. These hybrids are typically obtained together with large numbers of parental types. In habitats where the pure stocks are rare, no hybrids are obtained. The presence of hybrids only in the highly preferred habitats of the parental types can only be interpreted as a similar habitat preference and the displacement of the parental types by the hybrids. If one assumes an equal probability of producing hybrids, a reduction in hybrid vigor could be selectively advantageous to the population. That is, each vigorous hybrid would displace a parental type whereas the feeble hybrids would be displaced by members of their parental taxa. As a consequence, the number of breeding adults in the two

areas would differ and the difference would be associated with the relative vigor of the F_1 hybrids. During subsequent reproductive intervals, the production of young in a riffle would be inversely associated with the vigor of the hybrids. There also would be a strong tendency for the more numerous young to invade the riffle inhabited by the populations producing vigorous hybrids. Therefore, the populations with reduced hybrid vitality due to genetic factors would have a selective advantage over those that produced vigorous hybrids.

Two other ecologic circumstances may be involved in the requirements for selection of postmating reinforcement. The first would be stable adult populations and migratory young, a situation clearly applying to most darter populations. A riffle extensively collected for breeding adults soon becomes depopulated although young abound in the same habitats. These young could, of course, have been produced locally, but the numerous young occupying new stream channels after floods, etc., must have come in from elsewhere. Perhaps the best example may be provided by a series of collections from the North Llano River at Junction. Adults were obtained for laboratory experiments in November, 1963, at the start of the breeding season. Other collections indicated that the eggs obtained were those first to ripen that season. Although adults abounded in all riffles, the one that was collected most intensively yielded more than two hundred adults. On all subsequent collections this riffle was re-examined and a total of fewer than ten adults was obtained during the remainder of that season. All samples contained numerous young of all sizes, indicating continuous immigration from adjacent riffles. Many adults were collected in each sample from each adjacent riffle, even those separated by less than twenty meters of quiet water. The next year early collections were distributed more evenly and all riffles contained breeding adults throughout the season. Adult darters therefore tend to remain within limited areas and dispersal occurs during larval or young stages. This is not surprising because it is typical of many vertebrates.

The second ecologic circumstance that might relate to the selection model is genetic uniformity within individual populations. If each riffle were to maintain a high population density, considerable genetic diversity should occur on each; this is not likely to occur, however, in semiarid regions such as Central Texas where numerous natural catastrophes occur. Some localities are subject to repeated floods and after each flood most fish populations are reduced in number. Similarly, droughts may be severe and prolonged, radically reducing surface flow and as a consequence the numbers of fish. The populations could resume their former abundance upon re-establishment of suitable conditions and by chance would be expected to be relatively uniform genetically. The first catastrophe might set up the difference in hybrid potential, and those populations producing less vigorous hybrids would spread into other areas. Soon the populations might be mixed with regard to selection for

reduced hybrid vigor; but since natural catastrophes are seldom widely spaced in Central Texas the populations would be repeatedly reduced to minimal levels. Each reduction would produce many homogeneous populations and those with factors reducing hybrid vigor would be selected for following each catastrophe. Obviously, strong selection would be closely linked with frequent natural catastrophes. It is not surprising, then, that the best example of reinforcement can be found in fishes inhabiting catastrophe-prone semi-arid streams.

The only distinct support for posthatching reinforcement lies in the logperch \times greenthroat darter hybrids. Perhaps by coincidence these two species occupy similar ecologic niches (upper waters near the surface film) during the larval stages that were used for analysis of hybrid survival. Both *E. spectabile* and *E. punctulatum* occupy bottom habitats, and *H. shumardi* and *P. caprodes* occupy upperwaters during the same periods; their hybrid survival also indicates posthatching reinforcement.

Patterns of Hybrid Survival—Introgression. If introgression were to have a major role in selection of darters, one would expect to find an increase in hybridization potential of sympatric populations. None of the comparisons are distinctly more viable if sympatric.

Likewise, introgression would require maintenance of some degree of hybrid fertility. Most darter hybrid combinations are sterile and those that have some fertility typically have much less than do comparable controls. As a consequence, transfer of genetic material between distinct biological species would be difficult.

The only example of reasonably high hybrid fertility is between allopatric species. If these taxa were to exchange genes it would not result from the breakdown of an intrinsic isolation mechanism, and would merely reflect secondary contact between morphologically distinct races.

In summary, introgression does not seem to apply to darters.

Patterns of Hybrid Survival—Mutual Coexistence. There is little question that most speciation does involve geographic separations and if so, there should be a variety of levels of divergence. Therefore, it is unlikely that one series of tests would necessarily show strong evidence for isolation mechanisms in geographic races, which should be most apparent in premating situations.

The intraspecific patterns of hybridization potential show that *E. lepidum* is speciating in geographic isolation. Other intraspecific hybrids may have reduced fertilization potential; however, it is no more noticeable in within-system than in between-system hybrids. If geographic isolation were to be of extreme significance the intraspecific results should show considerably reduced fertilization percentage with increased allopatry. Although the above seems easily applicable to *E. lepidum* racial variation, this may not be a good

example of mutual coexistence. If the races are in the process of dividing into distinct species, their interactions should parallel inter-species interactions; however, the hybrid viability is distinctly not heterotic as is typical of interspecific hybrids. Obviously those incipient species have more gametic isolation than do "standard sympatric" pure breeding units; perhaps the hybrids would be heterotic when the species are sympatric and behavioral isolation mechanisms are well developed.

Patterns of Hybrid Survival—Proximate Inhibition. A large number of hybrid combinations exhibit an unexpected pattern of survival indices. This is the heterotic survival of hybrids between parents from distant or identical localities and the corresponding inhibition of hybrids between parents from two localities within the same stream system. There is some evidence that the converse may occasionally occur; however, the probabilities are relatively low and the phenomenon is less frequently observed. The best example of proximate inhibition is Colorado River *E. lepidum* females \times *E. spectabile* males. All hybrids but those from two different localities in the Colorado System are heterotic and deviation from the control has a probability of less than 0.001 of being by chance in all but the small sample using males from the Illinois River. Moreover, the reduced vitality of the hybrids from two Colorado localities also has a probability of less than 0.001 of being by chance. Therefore, it is exceedingly unlikely that the whole pattern has resulted from chance occurrence. The low fertilization (time lapse) potential of Colorado River *E. lepidum* sperm exposed to other Colorado System *E. spectabile* eggs is distinctive, significant, and follows the low survival potential of the reciprocal. All of these greenthroat \times orangethroat Colorado River hybrids are based on large samples from several localities and each shows the same phenomenon, indicating that it is not an isolated example. Perhaps of more significance is the presence of the same pattern in all combinations of the *E. caeruleum* \times *E. spectabile* hybrids from two different localities in the Mississippi-Missouri system (both Loose Creek and White River); Colorado System *H. scierus* \times *P. caprodes* hybrids; and Colorado System *P. caprodes* female \times *E. spectabile* male hybrids. This pattern is obviously widespread, both phylogenetically and geographically. Its frequent occurrence in the Colorado System experiments is undoubtedly caused by the more extensive use of Colorado stocks for tests so that individual experimental error is unlikely to mask selective results. Moreover, the species in which the pattern of proximate reduction of hybrid vitality is most apparent are also those most intensively studied, all of which indicates its widespread occurrence. This totally unexpected occurrence has misled me previously. In 1961, I pooled all within-Colonrad oSystem stocks in my report of *E. lepidum* sperm vitality. The drastically reduced fertilization potential of proximate populations combined with the reasonably high potential of sympatric hy-

brids caused an overall reduction of fertilization potential within the system so that reinforcement was reported. Separation of the Colorado stocks into proximate and identical localities shows that reinforcement *per se* does not explain the results.

The frequency of proximate inhibition indicates that it must be significant in interspecies interactions; however, it cannot easily be adapted to any of the three previously discussed patterns of interspecies interactions: introgression, reinforcement, or mutual coexistence. Clearly mutual coexistence cannot apply because if it did, no pattern should appear. Superficially a mixture of introgression and reinforcement seems to occur. That is, introgression within the stream system and reinforcement between systems. It is obvious that simultaneous action of introgression and reinforcement would tend toward equal results regardless of the level of sympatry. This does not occur here so one must examine the data to determine if another explanation is available. The within-system introgression is perhaps more apparent than real. Introgression would also require a reasonably high hybrid fertility. The *E. spectabile* \times *E. lepidum* hybrids are partly fertile and by extrapolation it seems likely that the *E. spectabile* \times *E. caeruleum* hybrids also are partly fertile. The available *H. scierus* \times *P. caprodes* hybrids show no sign of mature gametes and those with "male phenotypes" do not father young when stripped. Finally and most conclusively, the *E. spectabile* \times *P. caprodes* hybrids are not sexually dimorphic and the gonads are minute. It is inconceivable that these hybrids would be other than a dead end eliminating any selective value resulting from introduced foreign chromatin.

There is no reason to suspect that between-system reinforcement does not apply. If so, the apparently contradictory increase in hybridization potential of sympatric stocks must be examined carefully. If some other series of isolation mechanisms were to prevent hybridization of sympatric populations, and these mechanisms were relatively ineffective in isolating allopatric populations, the apparently contradictory reversal of hybridization potential would make sense. Almost every author who has discussed isolation mechanisms (C. L. Hubbs, 1961; Mayr, 1963; Mecham, 1961; Stebbins, 1950; etc.) considers that premating mechanisms are more effective than postmating mechanisms. Similarly, among the premating mechanisms those relating to courtship behavior usually are considered the most effective. Therefore, if a behavioral mechanism were to prevent sympatric mating and its effect were negligible in allopatric matings the theoretical requirements would be attained. Preliminary tests indicate that behavioral mechanisms operate much more effectively on sympatric than on allopatric populations. If so, the only natural way by which behavioral isolation mechanisms might break down would be if a darter was displaced to another locality within its stream system. Such a circumstance is precisely that to which proximate inhibition would apply.

A variety of data supports the above hypothesis. Because darters are known to break up into a variety of morphologic (Strawn, 1961; Hubbs, 1958b; Hubbs and Delco, 1960; Hubbs and Johnson, 1961) and physiologic races (Hubbs and Armstrong, 1962; Hubbs and Strawn, 1963) and these races can be separated by exceedingly short distances (Hubbs, 1964a), racial variation exists that might provide the differential behavioral responses. Moreover, hybridization in fishes is most likely if one species is abundant and the other rare (C. L. Hubbs, 1955, 1961). The application of light stimuli (a behavioral response) to the differential population density hypothesis for hybrid production was discussed by Hubbs and Martin (1965). The available evidence indicated that hybridization was most likely to occur when an upstream species was displaced downstream by a flood into the range of the second species. Not only would floods establish differential population densities; they would also reduce the effectiveness of light stimuli as isolation mechanisms. One of the classic hybrid swarms between *Gila* and *Siphateles* (Hubbs and Miller, 1943) clearly resulted from one species being introduced into the range of another. A darter hybrid swarm in the Guadalupe River clearly resulted from an upstream species (*E. lepidum*) being washed downstream into the area of a related species (*E. spectabile*). Therefore, proximate inhibition seems to supplement a behavioral mechanism and occurs when the two populations might get together in a situation where the finely tuned behavioral mechanism would not be effective. Obviously the populations inhabiting different stream systems would be unlikely to be mixed naturally and would not be involved in this problem.

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