

small fraction of the shell. In thin section the structure of this aragonitic unit is seen to be prismatic, with a feathery arrangement of the prism axes similar to that described by Bøggild (6) for other shell groups. The amino acid composition of the organic matrix from this unit is distinctly different from that of the nacreous and the two calcite layers. It has relatively fewer glycine and alanine residues and more of most of the other amino acids. It has a relatively higher number of both acidic and basic residues with an excess of basic residues.

The inner ligament superficially appears to be entirely organic material but is actually more than two-thirds aragonite and less than one-third organic matrix. The amino acid composition of this unit is most unusual, with nearly one-third the total residues made up of methionine. The high proline content is also distinctive. Perhaps the most unusual feature of the composition of this unit is the complete absence of tyrosine. In three separate samples ranging up to 3 mg of protein no tyrosine was detected in any of the records.

The unmineralized outer ligament differs markedly in amino acid composition from the matrix of the inner ligament. Proline and methionine are both much lower, while glycine makes up nearly 40 percent of the total. The ratio of acidic to basic residues is significantly lower than for the calcified units.

The periostracum, also unmineralized, shows greater variations in amino acid composition than any of the other structural units. The values for the periostracum given in Table 1 are from a single sample (taken from the growing edge opposite the hinge). Samples from the growing edge around the periphery of a single specimen may vary from 10 to 15 percent in the number of residues of many of the amino acids. The physical appearance of the periostracum also varies considerably around the periphery of the shell. Profound changes occur in the periostracum composition during the growth of very small shells (15 mm). After a shell length of 70 to 80 mm is attained, relatively few further changes occur. In shells of 20 mm in length, for example, leucine is higher by nearly a factor of 10, while tyrosine is lower, by a factor of two, than the corresponding values for a larger specimen, as listed in Table 1. During these changes the ratio of acidic to basic amino acids remains low, apparently a characteristic of the noncalcified components.

In comparing the amino acid compo-

sition of the nonmineralized components with the matrix from the mineralized fractions, the most obvious difference seems to be in the relative number of acidic and basic amino acid residues. The inner ligament, which has a high organic matrix content (30 percent), has the lowest ratio of acidic to basic residues of any of the calcified units. The nonmineralized units have still lower ratios.

The role of the organic matrix in mineralization is probably to provide a set of highly specific templates which act as the sites for the nucleation of the mineral phase (7). Certain side groups in the protein matrix may concentrate Ca^{++} and CO_3^{--} in specific positions and thus provide an appropriate initial concentration of these ions to nucleate the mineral phase. Aspartic and glutamic acid side chains could provide negatively charged sites, which would attract calcium ions. Similarly, the basic side chains could provide sites for the concentration of carbonate (or bicarbonate) ions. The fact that the nonmineralized units have very few acidic residues may indicate a possible mechanism for preventing mineralization.

A comparison of the compositions of the aragonite and calcite matrices shows the calcite matrices to be consistently higher in the ratio of acidic to basic residues. This may indicate the presence of a mixture of protein components, one of which may be rich in the basic amino acids. That the organic matrix of some shells is indeed a mixture of different proteins has been shown by Grégoire *et al.* (8) and more recently by Tanaka *et al.* (9).

The specificity of the organic matrix for the formation of aragonite or calcite has been considered by numerous workers. Differences in composition of the respective organic matrices have been detected by Roche *et al.* (10) and Tanaka *et al.* (11). On the other hand, Grégoire (5) has demonstrated the presence of organic fragments of identical microstructure in calcite and aragonite from *Mytilus edulis*, and Beedham (12) could not detect any significant differences in the amino acid composition of the aragonite and calcite matrices of *M. edulis*. Recent studies (13) show that the differences in the aragonite and calcite matrices of *M. edulis* are of the same kind and degree as those of *M. californianus* listed in Table 1.

The present data indicate a consistent difference in composition between the organic matrices of aragonite and calcite as well as between the different

structural units of aragonite. Further work should indicate the significance of these differences in the formation of the various shell structures and mineral phases (14).

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Resistance to DDT in the Mosquito Fish, *Gambusia affinis*

Abstract. *Mosquito fish from waters near cotton fields that have had a long history of treatment with chlorinated hydrocarbon pesticides exhibited a marked resistance to DDT compared with fish from areas which had had no past exposure to insecticides.*

Among vertebrates the fishes are notable for their susceptibility to chlorinated hydrocarbon pesticides. Odum and Sumerford (1) obtained an LD_{50} for mosquito fish (*Gambusia affinis*) at 0.01 part of DDT per million (ppm DDT). On the basis of published toxicity data, at 0.1 ppm DDT all fish will die within 12 hours, at 0.01 ppm only a few fish will be able to survive, and at 0.005 ppm many but not most test fish will be killed (2).

Resistance to DDT, although quite

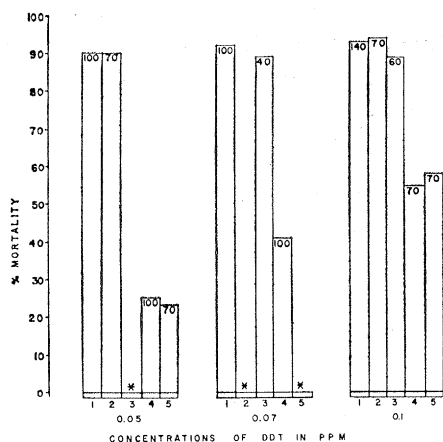


Fig. 1. Mortality of mosquito fish maintained 72 hours in three concentrations of DDT. Bars 1, 2, and 3 represent fish from untreated areas; bars 4 and 5 denote fish collected near treated cotton fields. Size of sample for each test is the number near the top of each bar. The asterisks indicate no specimens.

common among insects, was only recently demonstrated in vertebrates when Boyd, Vinson, and Ferguson (3) showed that two species of cricket frogs (*Acris*) were resistant. During that study, mosquito fish were occasionally found in pools and ditches completely surrounded by cotton fields that were heavily treated with chlorinated hydrocarbon insecticides, especially DDT. Insecticides were certain to have contaminated these places by intentional application, drift, drainage, or some combination of these methods of entry. Survival of mosquito fish in these adverse environments strongly suggested possible resistance to DDT.

In the present study, DDT susceptibility of fish from localities with a past history of exposure to DDT is compared with that of fish from previously untreated areas. Mosquito fish were collected from two treated sites, drained separately, near Sidon, Leflore County, Mississippi. Collections of fish from untreated areas were obtained from ponds near State College and 6 miles north of Starkville (Oktibbeha Co.), and near Aberdeen (Monroe Co.). After preliminary studies to establish the concentrations of DDT to be used, 1175 fish were tested; of these 185 were controls (110 from treated and 75 from untreated areas). The numbers of fish tested at each concentration are shown near the top of each bar in the graph (Fig. 1). Fish used in experiments ranged between 20 to 25 mm in length, and males outnumbered females three to one.

The DDT (4) was recrystallized from ethanol, weighed, and dissolved in acetone (reagent grade) to obtain a 0.1 percent solution. This was diluted in acetone to give 0.01, 0.007, and 0.005 percent solutions. One milliliter of each dilution was pipetted into a liter of water, so that the concentrations of the DDT in water were 0.1, 0.07, and 0.05 ppm. The liter of test solution was divided into 500-ml portions and placed in quart jars, and five fish from a single location were placed in each jar. An identical procedure with acetone only was followed for the control fish. All fish were maintained in the laboratory for 24 hours before they were tested. Mortality was recorded at 12-hour intervals for 72 hours. The room temperatures remained fairly constant throughout the experiments.

In Fig. 1 the percentage mortality is shown for each concentration of toxicant for all fish tested. The losses of fish for untreated areas (bars 1, 2, and 3) are strikingly higher than those of fish from the treated areas. Nearly identical mortalities occurred among populations that had similar past histories of exposure to insecticides. Thus, data on killing for the two treated collecting sites were similar, and those for the three untreated sites were also nearly identical.

The percentage mortality at each concentration for all treated and untreated areas was compared by means of chi-square tests at the level of 5 percent significance. All of the comparisons of differences in mortality between every possible combination of the treated with the untreated areas proved to be significant.

Differential mortality with respect to sex was not evident. The mortality among control animals was 10 percent from treated and 9.4 percent from untreated areas. The fact that fish were not fed during testing, the presence of acetone, or a change in environment may have produced these relatively high values.

The foregoing experiments indicate a resistance to DDT in mosquito fish bred in areas that have a long history of insecticide applications. The possible mechanism for development of resistance through selective pressure of insecticide and differential mortality in fish populations is implied in many field observations. For example, in a recent list of 75 cases of fish killed ostensibly because of agricultural poisons, there were only 12 cases where all fish

present were killed (5). If surviving fish in the other 63 cases are assumed to have genotypes that conferred a resistance to the toxicant, one could easily imagine that a genetically resistant population might result from periodic applications of insecticide (6).

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Illinoian and Wisconsin (Farmdale) Drifts Recently Exposed at Rockford, Illinois

Abstract. *New exposures in Rockford, Illinois, show fresh till overlying ferruginous gravel of marginal glacial origin. These, together with older sections, now slumped or no longer exposed, offer convincing evidence of two glaciations of the Rockford area, the first of Illinoian age and the second of early Wisconsin (Farmdale) age. The sections further suggest that the greater part of the drift fill in Ancient Rock River Valley is Illinoian, and that this is overlain by a relatively thin mantle of Farmdale drift.*

New exposures in the eastern part of Rockford, Illinois, show superposition of two glacial drifts of different ages, the lower one Illinoian in age, the upper one Wisconsin (Farmdale). They occur along the west and south sides of the Spartan Store (SW¼ NW¼ NW¼ section 29, township 44N, range 2E) (Fig. 1). The cuts are 500 feet south of U.S. route 20.

The two cuts afford a three-dimensional view. On the west side of the store the face is about 150 feet long and 25 feet high. As shown in Fig. 2, Farmdale till, grayish in color, overlies