NOTES

ELECTROPHORETIC ANALYSES OF HEMOCYANINS FROM FOUR SPECIES OF MUD CRABS, GENUS *PANOPEUS*, WITH OBSERVATIONS ON THE ECOLOGY OF *P. OBESUS*

The mud crab, known until now as *Panopeus herbstii* H. Milne Edwards, s.l., of the family Xanthidae, has been regarded as a common intertidal species throughout much of its range in the western Atlantic. As such, it commonly has been used in ecological, physiological, and genetic studies (reviewed in McDonald 1977). Four morphological forms (Rathbun 1930) are recognized as species in the paper by Williams (1983), their ranges outlined, and habitat preferences shown.

As part of the study on the genetic control of hemocyanin production, we examined several hundred individuals from coastal North Carolina and found considerable evidence that two of these four forms represent separate gene pools. Additional field observations revealed that these two forms occupy distinct areas in the intertidal zone, exhibit behavioral differences, and consume different prey. However, morphological differences other than color appeared to be slight. Smaller collections of *Panopeus* were made at Charleston, S.C., Big Pine Key and St. Petersburg, Fla., and Grand Terre Island, La. These included individuals representing the species *P. herbstii* H. Milne Edwards, s.s., *P. lacustris* Desbonne, *P. obesus* Smith, and *P. simpsoni* Rathbun. Electrophoretic analyses of their hemocyanins provide data that are compatible with taxonomic treatment of each as a distinct gene pool.

Materials and Methods

Crabs were collected by hand at low tide from the intertidal zone. Although ecological studies were confined to the area around Pivers Island, Beaufort, N.C., additional material was collected at Harkers Island and Swansboro, N.C., and at the localities mentioned above. Crabs from Big Pine Key and Grand Terre Island were shipped by air to Beaufort. The remaining crabs were bled at the collecting sites and hemocyanin samples transported on ice to Beaufort. Electrophoretic analysis of hemocyanins was performed following dissociation to monomeric subunits according to the methods of Sullivan et al. (1974) and Sullivan and Tentori (1981). Stomach analyses were made by injecting 1 ml of Formalin[1] into the cardiac region of the crab at the time of collection, and later, following its removal, contents of the cardiac stomach were examined under a binocular dissecting microscope.

Results

In studying the electrophoretic patterns of hemocyanin from the forms of *P. herbstii*, s.l. (Fig. 1), we associated an aberrent pattern with the color morph now recognized as *P. obesus* (Williams 1983). Conditions of preparation and electrophoresis of hemocyanins cause the polymeric hemocyanin to dissociate into subunits (Sullivan et al. 1974). The patterns shown in Figure 1 represent monomeric hemocyanin subunits. Six loci are active in a fiddler crab, *Uca pugilator* (Bosc), but the polypeptides of-

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

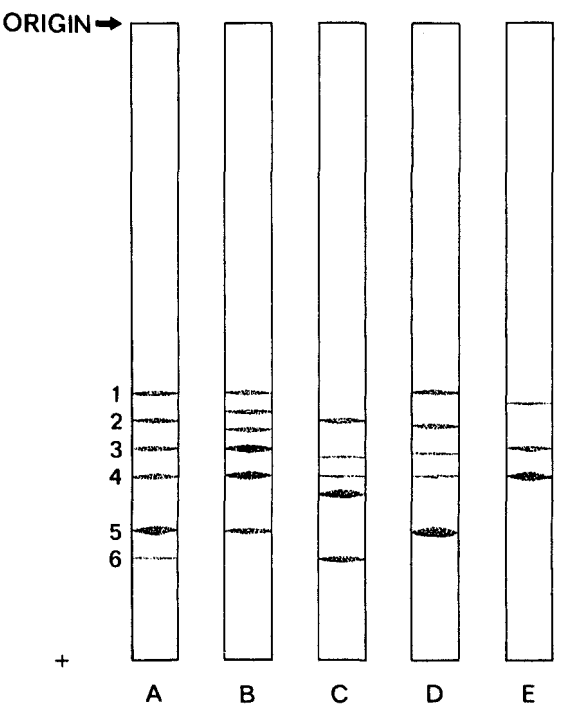

FIGURE 1.—Electrophoretic patterns of monomeric hemocyanins from *Panopeus herbstii*, s.s. (A), *P. obesus* (B), *P. lacustris* (C), *P. simpsoni* (D), and *Uca pugilator* (E) for comparison. Only one of several phenotypic patterns is shown.

ten overlay one another (Sullivan et al. in press). By comparison, in *P. herbstii*, s.s., at least six loci are also present with multiple alleles segregating at many of these loci (unpubl. data). The anodal zone of *P. obesus* differs from *P. herbstii*—most *P. obesus*' anodal zones (35 of 37 crabs examined) usually contain a single band, while in *P. herbstii* two bands are present in this zone (bands 5 and 6, Fig. 1). When double bands are present in *P. obesus* (because the individual is heterozygous at locus 5), the extra band does not align with band 6 but moves between bands 4 and 5. We are able to differentiate *P. obesus* from *P. herbstii* in every case by color pattern and by hemocyanin pattern. The hemocyanin pattern is extremely variable in both of these species, yet the differences described above were present in all individuals of *P. obesus* from Beaufort to St. Petersburg.

Electrophoretic analyses of the hemocyanins in specimens of *P. lacustris* ($n = 23$) from Big Pine Key, Fla., revealed five or six banded patterns which fell into seven phenotypic classes. This variable species shows a single major fast band, but it aligns with band 6 of *P. herbstii* instead of band 5. The remainder of the hemocyanin pattern is distinct when compared with those of the other forms (Fig. 1). Electrophoretic analyses of the hemocyanins in eight specimens of *P. simpsoni* from Louisiana reveals three phenotypic patterns which indicate polymorphism in this population also. Comparison of the patterns with those of *P. herbstii* reveals certain distinctions. A single fast band aligning with band 5 of *P. herbstii* is prominent in all gels. Band 6 is absent in all of the individuals of *P. simpsoni* which we examined. Comparison with *P. obesus* from the Carolinas and Florida reveals a similarity of electrophoretic mobilities but distinct intensity changes. In *P. obesus*, bands 3 and 4 are usually prominent; in *P. simpsoni*, band 5 is most prominent.

Our original samples ($n = 246$) of *Panopeus* from Beaufort, N.C., contained about 2% *P. obesus*. At Charleston, S.C., ($n = 38$) the frequency was 24%, and at St. Petersburg, Fla., ($n = 19$) it was 89% *P. obesus*. All individuals were collected in rocky areas or on oyster bars. Turner (1979) and Turner and Lyerla (1980) indicated that *P. obesus* was common in the upper intertidal at North Inlet, S.C., where the marsh grass, *Spartina*, grew in abundance. At Beaufort, N.C., our upper intertidal samples yielded virtually 100% *P. obesus*. A transect of an intertidal region containing both oyster rubble and *Spartina* revealed overlap for the two species only in the middle intertidal where oyster rubble and marsh grass were adjacent. *Panopeus obesus* was associated with burrows at the base of *Spartina* clumps; *P. herbstii* was in and under oyster shells. *Panopeus herbstii* alone occurred in the lower intertidal where only shells were present, and *P. obesus* alone was present in the upper intertidal where *Spartina* dominated; but in areas of lower salinity it appeared that *P. obesus* tended to displace *P. herbstii* on oyster bars.

Examination of adult females in June showed one ovigerous female out of five *P. obesus* whereas five of seven adult females of *P. herbstii* were ovigerous. Individuals of *P. obesus* appeared to live in burrows and would position themselves in the entrances to defend them. In general, *P. obesus* appeared more aggressive than *P. herbstii*. Stomach analyses of *P. obesus* revealed oyster spats (the primary food source of *P. herbstii*), shell and sea urchin fragments, and eggs and walking legs of *Uca pugnax* (Smith) and perhaps *Sesarma* sp., which are all primary food sources for *P. obesus*. Although the number of crabs examined was small, the ratio of the cheliped dactyl length (inner length, base to tip) divided by the carapace width averaged 0.25 for *P. obesus* and 0.22 for *P. herbstii*. If *P. obesus* does prey on other crabs in considerable numbers rather than mainly on oysters, one might expect a longer dactyl as compared with the oyster-feeding *P. herbstii*.

Discussion

Genetic variability at the hemocyanin loci in all populations of *Panopeus* which we have sampled complicates comparisons. Such variability is characteristic of many, but not all, temperate xanthid species (unpubl. data). Additionally, there are shifts in allelic frequencies in geographically separated populations, and the occurrence of local alleles is not unusual. However, in all areas where we have sampled two species, hemocyanin patterns can be designated as specific in spite of the "within-species" variability. Repeated sampling of the same individual over time has always yielded identical patterns, and the alleles at each locus are invariably in Hardy-Weinberg equilibrium.

We believe the evidence for the existence of two gene pools is very strong. In addition to hemocyanin data, Turner and Lyerla (1980) found unique alleles at the amylase, esterase, and malate dehydrogenase loci in the two South Carolina species. For instance, the most abundant amylase allele in *P. obesus* was not even present in adjacent populations of *P. herbstii*.

Habitat preferences are very clear in the Carolinas, at or near the northern limit of range for *P. obesus*. In western Florida, *P. obesus* is more abundant than it is in North Carolina and may occupy a broader range of habitats. Feeding habits in the two regions appear to

be dissimilar and breeding times may also differ. Detailed studies of the life histories of all the species in this complex are likely to reveal considerable differences. Unfortunately, the "forms" of *P. herbstii*, s.l., have been considered a single species and it is seldom possible to determine which "form" (= species) has been used in physiological, ecological, and behavioral experiments (McDonald 1977). The existence of four such similar species over a large range will undoubtedly provide an excellent opportunity for studies of their displacement and comparative biology.

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MUD CRABS OF THE *PANOPEUS HERBSTII* H. M. EDW., S.L., COMPLEX IN ALABAMA, U.S.A.

The mud crab, *Panopeus herbstii*, s.l. (sensu Rathbun 1930), occupies two distinct habitats in the Mobile Bay region of Alabama—the intertidal marsh and intertidal to subtidal oyster (*Crassostrea virginica* (Gmelin)) reef (Heard 1982). This paper presents an analysis of morphological attributes and ecological associations of these mud crabs, showing that the populations observed correspond to two sympatric species, *P. obesus* Smith and *P. simpsoni* Rathbun (Williams 1983).

Methods

Collection of mud crabs for morphological comparisons and feeding experiments was limited to 14 stations along southwestern Mobile Bay, Ala., and nearby eastern Mississippi Sound, from Dog River to Point of Pines, including Dauphin Island (Fig. 1), where *P. herbstii*, s.l., commonly occurs in a salinity range of 14 to > 20 ppt (May 1974). Figure 1 shows the location of stations which were sampled for crabs before destructive Hurricane Frederick struck in September 1978. The crabs, most numerous in waters with salinity >20 ppt, were sampled on four general types of substrate as follows: 1) Intertidal rubble (pieces of broken concrete over shell hash and silty sand at stations 1, 3, 4, 5, and oyster shell beach at station 2); collected by hand and in small mesh net from beneath pieces of cover. 2) Undercut marsh (mud eroded from beneath floating overhang of vegetation at edge of marsh leaving mat still attached to marsh sod at stations 6, 7, 8); overhanging mat was partly cut from bank with shovel and flipped over onto marsh to expose roots from which many small and some larger crabs were collected, mat then returned to water. 3) Mud bank (banks of hard mud