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SHORT COMMUNICATION

DIFFERENT HEMOCYANIN OXYGEN-AFFINITIES IN *CARCINUS MAENAS* FROM MAINE AND ARCACHON

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The green or shore crab *Carcinus maenas* (Linnaeus) is believed to have emigrated by ship from its native Europe to the Atlantic coast of North America. The chronological sequence of new records suggests the arrival in New York of pioneer individuals in the early 19th century, followed by gradual northward extension of the species range (Almaca, 1963; J. T. Carlton, personal communication). The number of immigrations and the present genetic relationships of various populations are unknown.

In an investigation of the CO_2 sensitivity of the hemocyanins (Hcs), we noticed (though did not report) that our O_2 affinity data for North American crabs collected in Maine (Mangum and Burnett, 1986) differed from those in the literature for European crabs collected in France (Truchot, 1973). While the magnitude of the CO_2 effect on oxygen affinity was essentially identical, the absolute values for the two populations (both control and experimental) were quite different. Differences in O_2 binding are expected when prior treatment, ionic composition of the solvent and temperature, for example, are not held constant. Our experience has been, however, that data for the same O_2 carriers, carefully collected under identical conditions, are indistinguishable, even when fundamentally different methods are employed (e.g. Mangum and Lykkeboe, 1979; Mangum and Burnett, 1986). Consequently, we have investigated the O_2 binding properties of the Hcs from North American and European representatives of *C. maenas* more thoroughly in order to examine the possibility of intrinsic differences.

To control for possible effects of thermal and/or seasonal acclimation, known in this and related species (Truchot, 1975; Mauro and Mangum, 1982), and of differences between the sexes (not detected by Mangum, 1990), we obtained females from Boothbay Harbor, Maine, in early March for comparison with our earlier data for males collected from Salsbury Cove, Maine, in July (Mangum and

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Burnett, 1986). As in our previous experiment, this group of crabs was fed and held in the laboratory at 18 °C and 33 ‰ salinity for a few days prior to sampling. In addition, freshly collected male crabs from the Atlantic coast of France near Arcachon were bled in July by K. I. Miller and J.-P. Truchot and the samples transported on ice by Dr Miller to our laboratory. These crabs were collected at a salinity above 30 ‰, thus precluding the possibility of salinity acclimation, which has been found in a related migratory species (reviewed by Mangum, 1990). In both cases O_2 binding measurements were completed within a few days of sampling and then the samples were frozen. Preliminary electrophoretic analysis (Mangum and Rainer, 1988) of subunit composition was later performed on these two groups of samples and also on previously frozen material from crabs collected in Germany and sent to us by C. R. Bridges and S. Morris.

Fresh samples were homogenized in a tissue grinder to remove the clot, centrifuged and then dialyzed against a physiological saline containing 464 mmol l^{-1} NaCl, 29 mmol l^{-1} Na_2SO_4, 20 mmol l^{-1} $MgCl_2$, 13 mmol l^{-1} $CaCl_2$, 11 mmol l^{-1} KCl and 3 mmol l^{-1} $NaHCO_3$. pH was adjusted by diluting (10 %) with the same saline plus Tris–maleate buffer (final concentration 0.05 mol l^{-1}). O_2 binding of both sets of samples was determined by the cell respiration method (Mangum and Lykkeboe, 1979). The results are also compared below with our earlier data, obtained using a tonometric method (Mangum and Burnett, 1986).

Regardless of method, season and sex, the data for Maine animals are homogeneous (Fig. 1). The slopes and positions of regression lines do not differ significantly, as indicated by broad overlap of 95 % confidence intervals. In contrast, the haemocyanin O_2-affinity of Arcachon crabs is uniformly lower than that of North American crabs (Fig. 1). The Bohr shifts are not significantly different; the slope of a regression line describing the combined data for North American crabs is -0.68 ± 0.09 (95 % confidence interval) and that for European crabs is -0.48 ± 0.16.

Cooperativity (n_{50}) appears to be the same (according to Student's t-test, $P>0.30$ for all possible comparisons of the three data sets in Fig. 1) in the North American and European crabs, although the non-linearity of the relationship between n_{50} and pH makes the reliability of this comparison more difficult to assess.

Thus, the present results indicate clear differences between the Hcs of Maine and Arcachon members of *Carcinus maenas* that are independent of sex, season and salinity. Since the waters are colder in Maine than in Arcachon, our control for season does not control entirely for temperature. However, warm-exposed individuals of this (and other) portunid species have higher O_2 affinities than cold-exposed crabs (Truchot, 1975; Mauro and Mangum, 1982), the opposite of the relationship observed here.

Mangum *et al.* (1988) and deFur *et al.* (1990) recently reported that prolonged hypoxia induces an intrinsic increase in the haemocyanin O_2-affinity of the related portunid *Callinectes sapidus*. We did not design our experiment to control for this possibility. However, hypoxic waters at a depth that might be encountered by

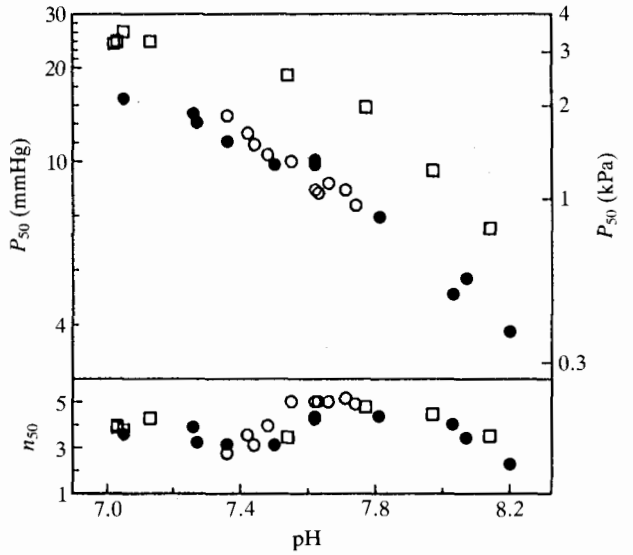

Fig. 1. O_2 binding of *Carcinus maenas* hemocyanin. Squares show data for Arcachon males collected in July; closed circles show data for Maine females collected in March (data obtained by the cell respiration method at 15°C). Open circles reproduce data reported by Mangum and Burnett (1986) for Maine males collected in July and obtained by tonometry at 16°C. In all cases the equilibration gases were scrubbed of CO_2.

C. maenas are unknown in Maine, and thus are highly unlikely to be responsible for the higher O_2 affinity.

The adaptive changes in O_2 affinity of *C. sapidus* Hc are brought about by shifts in the relative proportions of three of the 5–6 different polypeptide chains of the native Hc polymers (reviewed by Mangum, 1990). Our results show that the migration of two chains of Arcachon and Maine *C. maenas* haemocyanin differs repeatedly and in different kinds of electrophoresis. These differences are presently under more intensive investigation.

Although the environmental factors known to induce acclimatory changes in crustacean Hcs do not appear to be responsible for the present observations, we have no concrete evidence of genetic divergence. Nonetheless, we suggest that the Hcs of European adults of the species and their descendants in Maine can differ intrinsically.

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