of hummingbirds the greater the selection pressure they exert to produce suitably adapted plants and hence new races and species. This is the situation in the mountainous regions which are sites of high selection pressure on each of the species present, and is revealed by the sympatric nature of the hummingbird flower population. The allopatric population in lowland areas indicates a less intense selection pressure. A similar correlation of hummingbird dispersion and plant speciation has been found in Arizona, and is expected to occur in other areas over which the hummingbirds migrate.

Underground Shrimps

AMPHIPOD crustaceans are among the largest and least studied groups of North American freshwater invertebrates. It is therefore useful that a complete reappraisal of the systematics, speciation and distribution of the subterranean amphipod genus *Stygonectes* has been reported by John R. Holsinger in Bulletin 259 of the United States National Museum.

With 29 species, *Stygonectes* is the largest of the nine genera of Gammaridae. Mature adults range from 4·50 mm–19·50 mm; they lack eyes and pigments and inhabit subterranean areas such as caves and solution channels in limestone regions, and interstitial habitats where there is underlying non-cavernous rock. The genus has been divided into six species groups which are geographically distributed in the eastern United States, the south-central United States and central Texas.

The origin of *Stygonectes* is difficult to work out because of the complete lack of a fossil record and because of the absence of marine forms which can be regarded as related to an ancestral stock. Nevertheless, Holsinger has postulated a theory based on a series of freshwater invasions by ancestral marine stygonectic stock beginning early in the Cenozoic or even in the Upper Cretaceous. Ancestral stock was probably already inhabiting shallow coastal waters at this time and later passed through a transitional stage in brackish waters during periods of marine embayment. As the sea waters fluctuated, ancestral forms slowly migrated into freshwater areas lying adjacent to the old coastline. Finally, as the sea water permanently receded, they became tolerant of changing salinity and, as they established themselves in fresh water, moved slowly inland to occupy a whole series of newly created niches. Subsequent inland dispersal occurred through interstitial habitats developed in flood plains along principal rivers.

Three lines of evolution are indicated within the genus on the basis of morphology, geographic distribution and, to some extent, ecology. Patterns of insular speciation in the *emarginatus* and *spinatus* groups of *Stygonectes* in the central Appalachians, and in the *flagellatus* and *hadenoecus* groups of the Edwards Plateau, can be explained in terms of geographic isolation. Thus in the Appalachians, species in limestone-floored valleys were isolated from each other by high ridges of shales, sandstones and conglomerates. Similarly, ranges in the Edwards Plateau were isolated by barriers in the form of extensive faults and stratigraphic changes. Species with the widest range are found in the *tenuis* group and disperse freely through ground waters which are situated close to the surface. Some instances have been reported in Maryland of breakdown of ecological isolating mechanisms between two otherwise phenotypically and genotypically distinct species.

Dr Holsinger also considers recommendations for future study on the evolutionary biology of the genus. These include experiments to determine toleration to varying concentrations of salt water and improving methods to clearly delineate patterns of geographic variation.

Protein Protons

from our Molecular Biology Correspondent

THE past year has seen the burgeoning of proton magnetic resonance as a means of looking at the behaviour of particular functional groups in biological polymers. Some new examples in the current literature demonstrate again the scope and potential of the method.

Following on earlier work, Bradbury and Wilairat (*Biochem. Biophys. Res. Commun.*, **29**, 84; 1967) have examined the characteristic resonances of certain protons—specifically the C-2 ring protons—of the histidine residues in several proteins. It was shown previously that in ribonuclease, signals corresponding to different histidines could be distinguished. It is now shown that in hen's egg lysozyme the solitary histidine is clearly observable and that, in native insulin, cytochrome *c* and myoglobin peaks likewise show up in the expected region. On the other hand, in trypsin, chymotrypsin and their zymogens no histidine signals are detected until the protein is denatured. The simplest explanation, and the one offered by the authors, is that in the latter group of proteins the histidine rings are immobilized by interactions with their environment, whereas in the others they are presumably essentially free to rotate. Since protonation of the imidazole ring leads to a downfield shift, it is possible to follow its ionization curve, and Bradbury and Wilairat give the *p*K of the histidine residue of lysozyme as 6·3 in D_2O (corresponding to about 5·8 in H_2O).

The same experiment (with happily the same result) has been described by Jardetzky's group (Meadow *et al.*, *Proc. US Nat. Acad. Sci.*, **58**, 1307; 1967). In a human lysozyme these workers find the quite different, and anomalous, *p*K of 7·6. The addition to hen's egg lysozyme of a specific inhibitor leads to no detectable shift in the histidine resonance. In pancreatic ribonuclease, however, interesting effects are observed. In the first place, the 100 Mc instrument used by Meadows *et al.* makes possible the resolution of signals from all four histidine residues. Their hydrogen ion titration curves have thus been individually measured, and *p*K values of 5·6, 5·9, 6·1 and 6·6 ensue. Further, it is known that two histidines, his-12 and his-119, flank the active site, and their chemical modification annihilates the activity of the enzyme. Progressive addition of the competitive inhibitor, cytidine monophosphate, leads to quite large shifts in the signals from first one and then another of the histidines, and ultimately the remainder also show appreciable effects. There is no doubt, however, that two of the residues are most strongly affected, and it seems highly probable that these are near the active centre.

A staphylococcal nuclease, which requires calcium for activity and has also four histidine residues, has been