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Genetic Differentiation in Populations of the Freshwater Amphipod *Gammarus minus* Say in a Karst Area

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SUMMARY

The genetic makeup of populations in a particular geographic area should conform, in part, to environmental structure. Three polymorphic enzyme loci in the amphipod *Gammarus minus* are geographically differentiated in gene frequencies in the mid-Appalachian Mountains of the eastern United States, and genetic breaks usually coincide with topographical features and stream divides. Areas with well integrated drainage systems have a high degree of genetic uniformity.

INTRODUCTION

One of the underlying ideas in evolutionary biology is that a highly structured environment with well delineated dispersal barriers and routes of migration should profoundly affect the genetic makeup of the populations within it. Thus, genetic patterns of an aquatic organism should correlate well with drainage patterns and local hydrology of a given geographic area. This study examined two phenomena: areas of change and an area of great uniformity in the regional pattern of allozyme loci in the amphipod crustacean *Gammarus minus* Say in a karst area of southwestern West Virginia. This was done to see if the areas of change correspond to presumed dispersal barriers. Also the potential is great for a species to maintain gene flow throughout a karst area by dispersal through interconnected caves and water filled solution channels (Culver, 1970, 1971; Holsinger, 1967, 1969; Holsinger & Culver, 1970; Poulson & White, 1969; Avise & Selander, 1972), such as in Davis Spring Basin (DSB).

Gammarus minus is well suited for such a study. Populations are highly differentiated in allele frequencies at from one to three of the loci under investigation. The species is limited principally to springs, spring runs, and cave streams of limestone areas. Also, since it has no specific migratory stage in its

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life history, populations are semi-disjunct and partially isolated from others, even in local drainage systems, and isolated to a greater extent from populations in other streams and other limestone areas.

Gooch and Hetrick (1979) report on a number of geographic areas in which populations showed sharp genetic changes in contrast to other areas which were genetically uniform. This study examines four of these areas in more detail to determine the extent to which hydrology influences these genetic patterns.

MATERIALS AND METHODS

We examined three allozyme systems coded by polymorphic loci (malate dehydrogenase, *Mdh-1*; phosphoglucose isomerase, *Pgi-1*; peptidase, *Pep-1*) using electrophoretic techniques on cave and spring populations in karst areas of southeastern West Virginia (Gooch and Hetrick, 1979). A computer plot of allele isofrequency lines for the most common allele at each locus ($Mdh\text{-}1^{d}$, $Pgi\text{-}1^{c}$, and $Pep\text{-}1^{d}$) was used to examine the areas. Isofrequency maps of alleles are adapted from computer-drawn contour diagrams using the Numerical Surface Techniques and Contour Map Plotting set of programs 1130-(X-11X) for the IBM 1130 computer.

The study area lies in that part of the Greenbrier Valley bounded on the north by Droop Mountain in Pocahontas County and extending south about 70 km into Monroe County, West Virginia. Karst topography is highly developed, especially in the Great Savannah karst of south-central Greenbrier County and in Monroe County south of the Greembrier River (Jones, 1973).

Areas and localities sampled are as follows (see Fig. 1): Northern Greenbrier area (NG): 1. Martens Cave, 2. Lobelia Spring, 3. Marthas Cave, 4. Taylors Spring; Spring Creek area (SC): 5. Route ≠ 219 Spring, 6. Buckeye Creek Cave, 7. Culverson Creek Spring, 8. The Hole Cave; Davis Spring Basin (DSB): 9. Coffman Cave, 10. McClungs Cave, 11. Benedicts Cave, 12. Davis Spring; Davis Hollow Basin (DHB): 13. Sinks of the Run Cave, 14. General Davis Cave; Southern Greenbrier (SGR): 15. Organ Cave, 16. Dixons Spring, 17. Spring South of Second Creek, 18. Burnside Branch Cave; Ordovician Limestone Belt (OLB): 19. Kitchen Creek Spring, 20. Pattons Cave Spring, 21. Zenith Spring.

RESULTS AND DISCUSSION

Figure 1 shows allele frequencies for the three polymorphic loci examined. Martens Cave and Lobelia Spring populations in the Lobelia section of Greenbrier limestone crop out on the west side of Droop Mountain, while Marthas Cave is in the main NE-SW section cropping out to the east. Droop Mountain is potentially a formidable dispersal barrier for populations on either side and might also tend to impede north-south dispersal. However, drainage of the limestone outcropping on the northwestern side of Droop

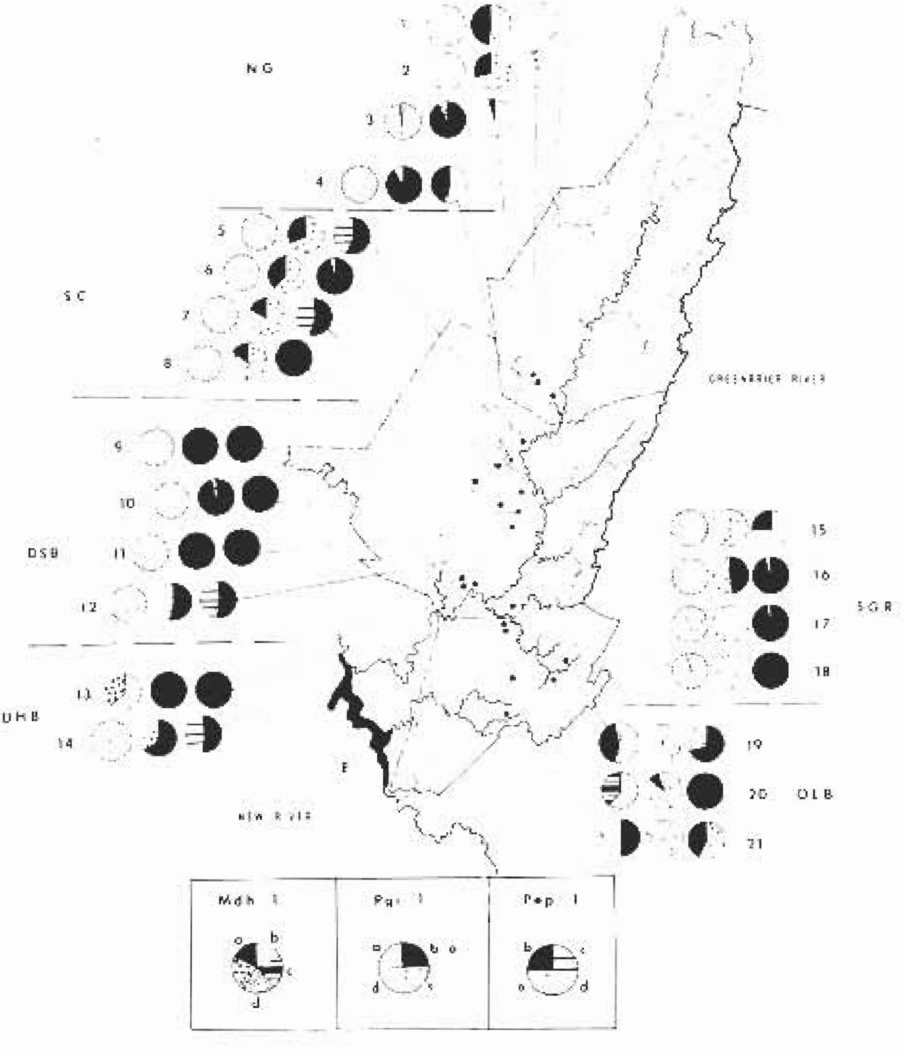

Fig. 1. Map showing location of sampled populations in three counties of southeastern West Virginia. Allele frequencies at the *Mdh-1*, *Pgi-1*, and *Pep-1* loci are represented respectively from left to right for each location and are indicated as percentages of circles.

Mountain flows under the mountain and a portion of it flows through the third Northern Greenbrier (NG) site, Marthas Cave, before resurging and flowing into the Greenbrier River (Wolfe, 1964; White and Schmidt, 1966). It is thus easy to envision amphipod dispersal under the spur of Droop Mountain. The hydrology, the *Pep-1* alleles that characterize this area (Fig. 1), and the bunching of *Pep-1*^{b} isolines at the area border (Fig. 2A) suggest that these populations have been relatively isolated from those of surrounding geographic areas. Also, the *Pgi-1*^{c} isoline patterns (Fig. 2A) outline the Droop Mountain barrier within (NG), separating the Martens Cave and Lobelia Spring populations from Marthas Cave on the main southeastern belt.

The Spring Creek drainage basin (SC) is another potential dispersal barrier representing the local base level of the water table and receives discharge of subsurface drainage. As described by Jones (1973), subsurface drainage of the area is divided into several sub-basins, namely Culverson Creek, Buckeye Creek, and The Hole Cave, all of which resurge into Spring Creek. Our experience and that of Holsinger and Culver (1970) strongly suggest that *G. minus* is absent from large streams like Spring Creek, except as occasional drifting individuals. However, *G. minus* is found in the springs where each of these sub-basins resurge into Spring Creek. These springs are located at almost equally spaced points along Spring Creek. This distribution of springs could aid in stepping stone dispersal of *G. minus* into various sub-basins and thus produce a pattern of genetic homogeneity within the Spring Creek drainage basin.

Populations within the Spring Creek drainage are relatively homogeneous for both *Pep* and *Pgi*. The isolines for *Pgi-1*^{c} are bunched slightly between NG and SC, but the frequency of *c* falls sharply almost to zero between SC and DSB (Fig. 1 and 2A). This genetic discontinuity coincides with the subterranean drainage divide between The Hole Cave, which discharges northeastward into Spring Creek, and the vast cave network of the DSB, draining southwest. The divide is only a few hundred meters wide (Jones, 1973 Mapa). Also, *Pep-1*^{b} shows a steepening of the cline near the NG-SC boundary, but, unlike *Pgi-1*^{c}, there is no corresponding break across the SC-DSB divide. These data suggest that Spring Creek itself is not an important dispersal barrier within the basin, although the basin as a whole appears to isolate areas to the north and south.

One of the most interesting and largest of the areas studied is the Davis Spring Basin (DSB). Its northern segment drains through Higginbotham's Cave and then through Coffman Cave. Coffman Cave has been dye traced to Davis Spring (Jones, 1973). Drainage in the eastern part of DSB is primarily subsurface through extensive, well integrated cave systems. Populations from a number of these caves were sampled. Although the caves have a dendritic pattern, their general orientation and water flow are southwest. Davis Spring appears to be the point of resurgence for this entire basin (Jones, 1973). Obviously this area has a well integrated subterranean drainage system, and the potential for dispersal and consequent gene flow is great. All populations of this area are genetically homogeneous at all three loci (Fig. 1). Both electrophoretic and hydrologic data lend considerable weight to the proposal that at

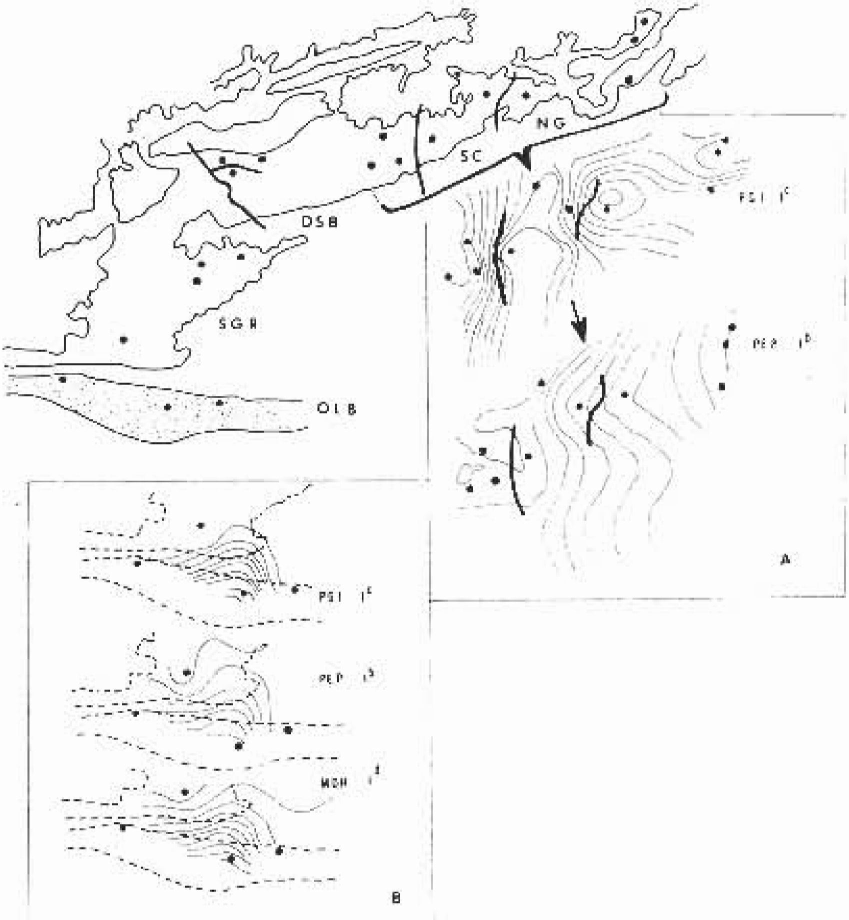

Fig. 2. Maps showing isofrequency lines of the most common alleles at each locus, respectively *Pgi-1*^{c}, *Pep-1*^{b} and *Mdh-1*^{d}. Isolines for areas NG, SC, and DSB boundaries are shown in map A, those for isolines between SGR and OLB in map B. Upper map is of the Greenbrier Limestone outcrop (solid-lined borders and open area) and the Ordovician limestone belt (solid-lined borders with stippling). Heavy lines represent boundaries of *a priori* defined areas.

least intermittent gene flow is occurring or has occurred between these populations.

The Ordovician Limestone Belt (OLB) consists of a narrow band of Ordovician limestone cropping out in the eastern part of Monroe County, West Virginia, occupying the valley floor on the west side of Peters Mountain and Back Valley (Davies, 1965). Two major surface drainage systems, Greenbrier River and New River, are found here. Both Kitchen Creek Spring and Patton's Cave Spring are within the Greenbrier drainage while Zenith Spring is in the New River drainage. Both alleles and frequencies vary considerably between sites. However, OLB populations exhibit a pattern of heterogeneity which corresponds with surface drainage patterns. All three loci demonstrate a high isoline density between OLB and Southern Greenbrier area (SGR) populations (Fig. 2B), indicating a high degree of genetic differentiation. The total pattern revealed by these data is not surprising since the limestone here is narrow, without a well integrated subterranean drainage system and is geologically isolated from the other portions of the study area on Greenbrier Limestone. The SGR-OLB barrier to subsurface connectivity is associated with the genetic discontinuity.

Populations have been examined within the James River drainage which are also geologically isolated from OLB by a series of clastic ridges. These populations are also sharply differentiated from OLB populations. This reinforces our conclusion that hydrology can markedly affect the genetic pattern of an area.

RÉSUMÉ

La composition génotypique des populations d'une aire géographique particulière serait partiellement fonction de la structure de leur environnement. Trois loci enzymatiques polymorphes de l'Amphipode *Gammarus minus* montrent des fréquences géniques présentant des différences géographiques dans la région moyenne des Monts Appalaches, à l'Est des Etats-Unis. Les discontinuités génétiques coïncident habituellement avec des éléments de la topographie ou les lignes de partage des eaux. Les aires où le réseau hydrographique est très cohérent montrent un degré élevé d'uniformité génétique.

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