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Cave beetle genetics: geology and gene flow

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The genetic structure of four species of obligate cave-dwelling carabid beetles, occurring in two plateau karsts, was examined using gel electrophoresis of proteins. Geological features, in particular streams and rivers, appear to be strong barriers to gene flow in both karst regions. Additional genetic differentiation among populations, which is not obviously related to geological features, occurs in both plateaus. As a result, gene flow levels in these species are generally lower than those reported for similar surface-dwelling species in the same geographical area.

Keywords: biogeography, carabid beetles, caves, gene flow.

Introduction

Approximately 250 species of obligate cave-dwelling (i.e. troglobitic) trechine beetles (Coleoptera; Carabidae; Trechinae) occur in caves of the southeastern United States (Barr, 1981). Cave speciation in these beetles has been modelled in three stages (Barr, 1985; Barr & Holsinger, 1985; Barr, 1990):

1 ancestral beetles living above ground gradually adopted a mode of life in deep soil, then became restricted to it;

2 these 'edaphobites', many of which lost functional eyes and melanin as soil dwellers, ultimately colonized caves;

3 as either edaphobites or troglobites, gene flow between local populations was greatly reduced and effectively restricted to limited subterranean dispersal.

During the Pleistocene, cool, moist periglacial climates are believed to have favoured broader geographical distributions of the pre-cave ancestors, whose ranges were subsequently restricted in warmer, drier interglacials to single caves or interconnected cave systems.

Populations of troglobitic trechines in different cave systems may be extrinsically isolated by (a) large rivers, or (b) non-limestone strata interposed between the caves (Barr, 1985). A substantial body of genetic data indicates that gene flow among populations of terrestrial troglobites, including some trechines, may be much lower than that observed in similar, surface dwelling species (Laing *et al.*, 1976a, b; Caccone, 1985; Kane & Brunner, 1986; Caccone & Sbordoni, 1987). The maximum extent of geographical ranges of cave trechines occurs in the two Mississippian plateaus (Fig. 1a) formed on widely exposed, massive-bedded, highly cavernous limestones. Stratigraphic barriers in the Mississippian plateaus are few, and the frequency of sympatry among two or more species is much higher than it is in cave regions of the heavily faulted Appalachian valley and ridge province (Barr, 1967, 1968, 1985). The Mississippian plateaus thus provide a high potential for subterranean dispersal and gene flow among conspecific populations of troglobites. A high dispersal potential in Mississippian karst regions permits not only more extensive geographical ranges for troglobitic species, but more sympatry, higher population densities, and greater community stability, in contrast to geographically limited ranges, infrequent sympatry, and irregular population fluctuations in the Appalachian valley (Barr, 1967).

Trechines in the Mississippian plateaus exhibit morphological variation over their ranges which is often correlated with potential dispersal barriers. The variation observed often involves subtle, but consistent, differences in minor morphological characters (Barr, 1979, 1985). Although such differentiation appears to reflect a reduction in gene flow across these barriers, it is often difficult to determine the degree of reduction from the morphological features alone. Furthermore, there is evidence to suggest that local populations of some troglobites may be highly differentiated genetically without exhibiting corresponding morphological differentiation (Laing *et al.*, 1976a, b).

In this study we examine some widespread species of troglobitic trechines in two Mississippian plateau karst regions (MP-I and MP-II of Fig. 1a; cf. Barr, 1985). Much of the morphological variation in these trechines appears to be associated with fluvial barriers and drain-

age divides (Barr, 1985), but some of the variation is not obviously related to such features. In some instances the presence of morphologically intergrading populations has been used to infer gene flow (Barr, 1979, 1985), with morphological races assigned subspecific status. In other cases lack of morphological intergradation has been assumed to indicate lack of gene flow, and the morphotypes are presumed to be distinct species (Barr, 1985). To assess quantitatively the efficacy of geological barriers to gene flow, we have examined several populations of these taxa using gel electrophoresis or proteins. Such data can be used to describe the genetic structure of populations and to estimate levels of gene flow among populations (Kane *et al.*, 1990). As a result they permit us to test hypotheses about gene flow and genetic differentiation derived from morphological and biogeographical data.

Methods

Species investigated

Multiple populations of four species of troglobitic trechines were sampled in this study. Three species, *Pseudanophthalmus barberi, P. stricticollis*, and *P. tenuis* occur in the northern portion of MP-I (Fig. 1c). All three species are similar but can be consistently distinguished on the basis of minor morphological characters (Barr, 1985). All are riparian predators, locally quite abundant on mudbanks along the margins of cave streams. *Pseudanophthalmus barberi*, in northern Kentucky, is isolated from *P. tenuis*, in Indiana, by the Ohio River, one of four known dispersal barriers in MP-I. There is no known dispersal barrier between the range of *P. tenuis* and *P. stricticollis* to the north-west, and at one point populations of the two species are separated by only 4 km. Despite this fact there is no morphological evidence of hybridization between the two taxa (Barr, 1985). Four populations of *P. barberi*, three populations of *P. stricticollis* and six populations of *P. tenuis* (Fig. 1b) were each sampled on one or more occasions during the period 1982–1985. The approximate distance between the northernmost *P. stricticollis* population (EL; Fig. 1c) and the southernmost *P. barberi* population (NE; Fig. 1c) is 118 km.

Darlingtonea kentuckensis, the other species studied, occurs in MP-II in southeastern Kentucky, with a single known Tennessee population (Barr, 1960; Fig. 1b). It is a large (7.0–8.0 mm), locally abundant and widely distributed trechine that has evolved specialized behaviour which allows it to prey on eggs of the cave cricket *Hadenoecus cumberlandicus* (Orthoptera; Saltatoria; Rhaphidophoridae) (Marsh, 1969). *D. kentuckensis* is differentiated over its range into at least

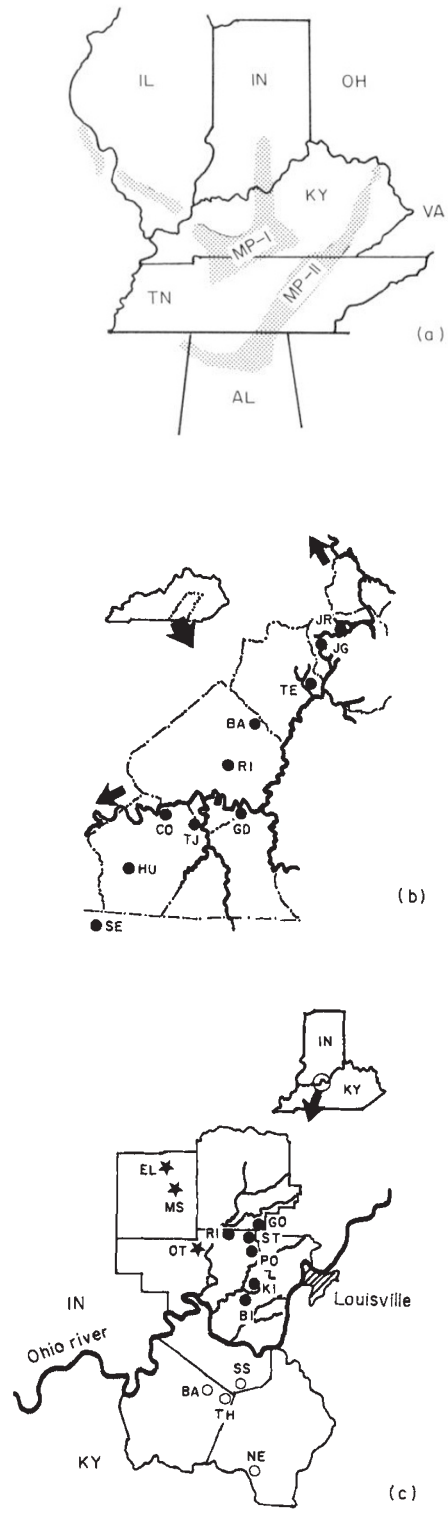

Fig. 1 (a) Map of the southeastern United States showing the location of the Western (MP-I) and Eastern (MP-II) Mississippian Plateaus. 1(b) Map of the collecting localities for *Darlingtonea kentuckensis* in Kentucky and Tennessee. 1(c) Map of the collecting localities for *Pseudanophthalmus barberi* (○), *P. stricticollis* (✱) and *P. tenuis* (●) in Indiana and Kentucky.

seven morphological races (Barr, 1985), with much of this variation associated with the major fluvial barriers that occur in the region. The drainage divide between the Kentucky and Cumberland river basins (Fig. 1b) is also the approximate dividing line between the two northernmost races of *D. kentuckensis*. In the southern part of the species' range, Cumberland River and its Big South Fork form a 'river triangle' (Fig. 1b), with different races in all three sectors (Barr, 1985). Ten *D. kentuckensis* populations (Fig. 1b) were sampled in 1984–1986 to include all seven putative morphological races and some populations which are morphological intergrades. The geographical extent of these populations (i.e. site JR to site SE; Fig. 1b) is approximately 148 km.

Electrophoresis

A total of 23 populations of trechines were collected. Beetles were transported alive in an ice chest to the laboratory at the University of Cincinnati where they were separated by species, sexed and stored individually in 400-μl centrifuge tubes at −80°C prior to electrophoresis. All electrophoresis was done on vertical polyacrylamide gels using a Hoefer Scientific SE600 System. Resolving gels of 5–7% acrylamide and 0.13–0.18% bisacrylamide, depending on the system being run, were maintained at 5°C during electrophoresis using a low temperature circulator. The three buffer systems used were:

1 stacking gel: 0.4 M Tris-phosphate pH 5.5; resolving gel: 0.57 M Tris-HCl pH 7.5; electrode: 0.008 M Tris-0.03 M barbituric acid pH 7.0 (Hames & Rickwood, 1981);

2 stacking gel: 0.5 M Tris-HCl pH 6.8; resolving gel: 3.0 M Tris-HCl pH 8.8; electrode: 0.05 M Tris-0.38 M glycine pH 8.3 (Hames & Rickwood, 1981); and

3 stacking gel: none; resolving gel: 1.5 M Tris-citrate pH 9.0; electrode: 0.09 M Tris-0.03 M borate pH 9.0 (Ortec, 1972).

Individual beetles were homogenized in 50 μl of grinding buffer [0.01 M Tris-HCl pH 7.0, with 0.001 M EDTA, 1 per cent (by volume) Triton-X, 25 per cent (by weight) sucrose], and centrifuged for 10 min at 4°C. This procedure yielded enough supernatant for four samples per individual. Thus, a minimum of four and, with multiple staining of some gels, a maximum of seven loci could be resolved per individual.

Nine enzyme systems encoded by 10–11 presumptive gene loci were consistently scored in all four species. These were aconitase (ACO) and esterase (EST) using buffer system 1; hexokinase (HEX), mannose phosphate isomerase (MPI), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM, two loci in *D. kentuckensis*, one each in *Pseudanophthalmus* spp.), and superoxide dismutase (SOD) using buffer system 3; and, malate dehydrogenase (MDH, 2 loci) and xanthine dehydrogenase (XDH) using buffer system 2. Staining techniques for these systems were adopted variously from Brewer (1970), Shaw & Prasad (1970) and Harris & Hopkinson (1976). Ten loci were resolved for the three *Pseudanophthalmus* spp. and 11 loci were resolved for *D. kentuckensis*.

The majority of the data analysis was accomplished using a Fortran 77 version of the BIOSYS-1 program of Swofford & Selander (1981). This program contains routines for population genetic analysis as well as procedures for the production of phenograms and for phylogenetic analysis. Gene flow levels were estimated using Wright's (1931) island model for gene flow (see also Slatkin & Barton, 1989). The island model permits estimation of N_m, the average number of migrants exchanged per generation, from F_{ST} using the relationship:

$$F_{ST} = 1/(4N_m + 1).$$

Results

Patterns of geographical variation

Overall levels of polymorphism (0.99 criterion) are similar in the four species studied. Nine of the 11 electrophoretic loci examined in *D. kentuckensis* (Table 1) have two or more electromorphs segregating within or among populations, and seven of 10 loci are polymorphic within or among populations of the three *Pseudanophthalmus* spp. (Table 2). Major differences arise, however, in the manner in which the genetic variation is partitioned within each group.

Nearly all of the genetic variation observed in *D. kentuckensis* is partitioned among populations ($F_{ST} = 0.963$; Table 3), with low levels of variation within populations ($H = 0.009$; Table 4). The UPGMA dendrogram of Rogers' Coefficient of Genetic Similarity (S) for the 10 populations (Fig. 2) indicates that much of the among-population variation is due to differentiation among races. There is a major break in similarity between the four northernmost and six southernmost populations. This break lies between the BA and RI populations (Fig. 1b) and occurs in the absence of any obvious geological barrier. The two clusters within the northern group of populations correspond to the Kentucky River (JG and JR) and Rockcastle River (BA and TE) drainage divide. Among the southern populations three branches, representing the northern (RI), southwestern (CO, HU, SE and TJ) and southeastern (GD) sectors of the 'river triangle',

can be recognized. Populations within races (e.g. JG and JR; TE and BA) show little differentiation. Furthermore, the SE population is not genetically distinct from other populations in the south-west (Fig. 2) despite being considered a distinct race morphologically.

There is substantial morphological and biogeographical evidence (Barr, 1985) for the derivation of

Table 1 Electromorph frequencies for 10 populations of *Darlingtonea kentuckensis*. Population designations refer to locations shown in Fig. 1b and *N* refers to sample size

	Population									
Locus	JG	JR	TE	RI	GD	TJ	SE	BA	HU	CO
1. Monomorphic loci with the same electromorph fixed in all populations: HEX-1; SOD-1										
2. Polymorphic loci										
ACO-1										
n	14	39	24	43	37	42	9	28	39	11
A	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000	1.000
EST-1										
n	36	69	51	68	48	54	10	32	39	17
A	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
B	1.000	0.993	1.000	0.000	0.000	1.000	0.950	1.000	0.756	1.000
C	0.000	0.007	0.000	0.993	0.000	0.000	0.050	0.000	0.244	0.000
D	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000
MDH-1										
n	22	29	28	47	37	48	6	28	39	12
A	1.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
B	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000
MDH-2										
n	17	25	23	47	37	45	6	28	39	12
A	1.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
B	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000
MPI-1										
n	13	45	20	45	39	49	10	39	63	19
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.947
PGI-1										
n	21	54	23	52	49	61	9	37	63	26
A	0.024	0.000	0.022	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.976	1.000	0.978	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1										
n	10	13	16	25	29	9	9	16	15	7
A	1.000	1.000	1.000	1.000	0.069	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.931	0.000	0.000	0.000	0.000	0.000
PGM-2										
n	10	13	16	25	30	9	9	16	15	7
A	1.000	1.000	1.000	1.000	0.033	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.967	0.000	0.000	0.000	0.000	0.000
XDH-1										
n	16	31	26	43	41	47	6	27	34	18
A	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2 Electromorph frequencies for 13 populations of *Pseudanophthalmus* spp. Population designations refer to locations shown in Fig. 1c and *n* refers to sample size

	P. barberi				*P. stricticollis*			*P. tenuis*					
Locus	BA	NE	SS	TH	EL	MS	OT	BI	GO	KI	PO	RI	ST
1. Monomorphic loci with the same electromorph fixed in all populations: MDH-1; SOD-1; XDH-1.													
2. Polymorphic loci													
ACO-1													
n	31	30	2	37	6	42	30	42	26	30	15	19	37
A	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000
B	0.016	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.968	0.717	1.000	0.973	0.917	0.929	1.000	0.917	0.981	1.000	1.000	1.000	0.919
D	0.016	0.267	0.000	0.027	0.083	0.071	0.000	0.071	0.019	0.000	0.000	0.000	0.081
EST-1													
n	35	36	12	65	9	43	38	46	48	21	11	19	47
A	0.014	0.444	0.000	0.031	0.000	0.023	0.000	0.011	0.010	0.000	0.000	0.000	0.032
B	0.043	0.542	0.000	0.023	0.278	0.174	0.000	0.011	0.063	0.143	0.136	0.132	0.085
C	0.943	0.014	0.917	0.923	0.722	0.802	1.000	0.978	0.917	0.833	0.864	0.684	0.745
D	0.000	0.000	0.083	0.023	0.000	0.000	0.000	0.000	0.010	0.024	0.000	0.184	0.117
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
HEX-1													
n	39	29	2	37	8	32	19	37	28	40	15	30	23
A	0.000	0.017	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.017	0.000
B	1.000	0.983	1.000	0.932	1.000	0.984	0.974	1.000	1.000	1.000	1.000	0.983	1.000
C	0.000	0.000	0.000	0.068	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2													
n	51	32	3	41	8	32	25	31	35	30	8	9	36
A	0.000	0.000	0.000	0.012	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	1.000	1.000	1.000	0.988	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI-1													
n	31	33	2	52	5	28	21	39	37	21	10	29	32
A	0.016	0.030	0.000	0.019	0.000	0.000	0.000	0.064	0.014	0.000	0.000	0.000	0.000
B	0.984	0.970	1.000	0.971	1.000	1.000	1.000	0.936	0.986	1.000	1.000	1.000	1.000
C	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGI-1													
n	40	40	4	48	7	37	29	52	51	40	12	30	33
A	0.012	0.000	0.000	0.000	0.071	0.027	0.017	0.029	0.000	0.000	0.000	0.000	0.000
B	0.063	0.025	0.000	0.073	0.786	0.838	0.655	0.125	0.824	0.050	0.750	0.667	0.591
C	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.900	0.575	0.875	0.875	0.143	0.135	0.328	0.798	0.157	0.813	0.250	0.333	0.409
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000
F	0.025	0.387	0.000	0.052	0.000	0.000	0.000	0.048	0.020	0.012	0.000	0.000	0.000
G	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1													
n	38	35	10	41	4	21	15	25	33	21	5	20	41
A	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024
B	0.053	0.000	0.000	0.024	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.329	0.029	0.300	0.341	0.125	0.143	0.033	0.000	0.015	0.071	0.100	0.000	0.098
D	0.592	0.957	0.650	0.524	0.625	0.857	0.967	0.980	0.955	0.929	0.900	1.000	0.866
E	0.026	0.014	0.050	0.098	0.000	0.000	0.000	0.020	0.030	0.000	0.000	0.000	0.012

Table 3 *F*-statistics and heterogeneity chi-square values for four species of cave-dwelling carabid beetles

Locus	F_{IT}	F_{IS}	F_{ST}	χ^2
D. kentuckensis				
ACO-1	−0.001	−0.012	0.011	5.82ns
EST-1	0.851	−0.147	0.871	1584.90***
MDH-1	1.000	—	1.000	592.00***
MDH-2	1.000	—	1.000	558.00***
MPI-1	−0.005	−0.056	0.048	34.10***
PGI-1	0.978	−0.023	0.979	779.45***
PGM-1	0.918	−0.074	0.924	272.90***
PGM-2	0.962	−0.034	0.963	287.60***
XDH-1	1.000	—	1.000	578.00***
Average	0.959	−0.103	0.963	
P. barberi				
ACO-1	0.232	0.085	0.160	30.98***
EST-1	0.523	0.017	0.515	246.69***
HEX-1	0.306	0.277	0.040	12.34ns
MDH-2	−0.003	−0.012	0.009	2.11ns
MPI-1	−0.017	−0.025	0.008	1.72ns
PGI-1	0.008	−0.153	0.140	93.52***
PGM-1	0.127	0.033	0.096	43.90***
Average	0.236	−0.004	0.239	
P. stricticollis				
ACO-1	−0.054	−0.084	0.029	4.62ns
EST-1	0.270	0.189	0.100	20.58***
HEX-1	−0.011	−0.023	0.012	2.96ns
PGI-1	0.097	0.062	0.038	8.67ns
PGM-1	0.366	0.283	0.115	21.03***
Average	0.201	0.138	0.074	
P. tenuis				
ACO-1	0.389	0.361	0.043	14.85ns
EST-1	0.091	0.034	0.060	55.62***
HEX-1	−0.003	−0.017	0.014	4.78ns
MPI-1	−0.013	−0.059	0.043	12.94*
PGI-1	0.283	−0.019	0.297	225.38***
PGM-1	0.086	0.052	0.036	20.31ns
Average	0.204	0.032	0.179	

ns = not significant; *$P<0.05$; ***$P<0.005$.

Table 4 Levels of polymorphism (P), heterozygosity (H) and average number of alleles per locus (A) in populations of four species of cave-dwelling trechine carabid beetles

Population	P	H	A
D. kentuckensis			
BA	0.000	0.000	1.0
CO	0.091	0.010	1.1
GD	0.182	0.019	1.2
HU	0.091	0.040	1.1
JG	0.091	0.004	1.1
JR	0.091	0.001	1.1
RI	0.091	0.001	1.1
SE	0.091	0.009	1.1
TE	0.091	0.004	1.1
TJ	0.091	0.002	1.1
Average	0.091	0.009	1.1
Overall	0.818		
P. barberi			
BA	0.500	0.091	2.1
NE	0.600	0.170	2.1
SS	0.300	0.092	1.4
TH	0.700	0.112	2.4
P. stricticollis			
EL	0.400	0.118	1.6
MS	0.500	0.090	1.7
OT	0.300	0.053	1.4
P. tenuis			
BI	0.500	0.061	1.9
GO	0.500	0.066	1.9
KI	0.300	0.068	1.6
PO	0.300	0.097	1.3
RI	0.300	0.079	1.4
ST	0.400	0.122	1.9
Pseudanophthalmus spp.			
Average	0.431	0.094	1.7
Overall	0.700		

the three *Pseudanophthalmus* spp. examined in this study from a common ancestor in the Pleistocene. The UPGMA dendrogram of the 13 populations (Fig. 3) suggests at least three evolutionary events. A major break in similarity occurs between the four Kentucky populations (i.e. *P. barberi*) south of the Ohio River, and the nine Indiana populations (*P. stricticollis* and *P. tenuis*) to the north (Fig. 1c), with near fixation of alternative alleles at the MDH-2 locus (Table 2) between the two groups. Despite the apparent lack of gene flow at present, however, common and rare electromorphs of the same mobility occur in populations on both sides of the river at other loci (e.g. EST, PGI, PGM; Table 2).

The other two evolutionary events (Fig. 3) occur among the *barberi* and among the *tenuis* populations, respectively. The NE population is distinguished from the remaining three *barberi* populations in the absence of any obvious dispersal barrier other than distance. The differentiation of the BI and KI populations from the other four *tenuis* populations further north (GO, PO, RI, ST) shows geographical clustering. Differentiation is most apparent at the PGI locus (Table 2). The BI

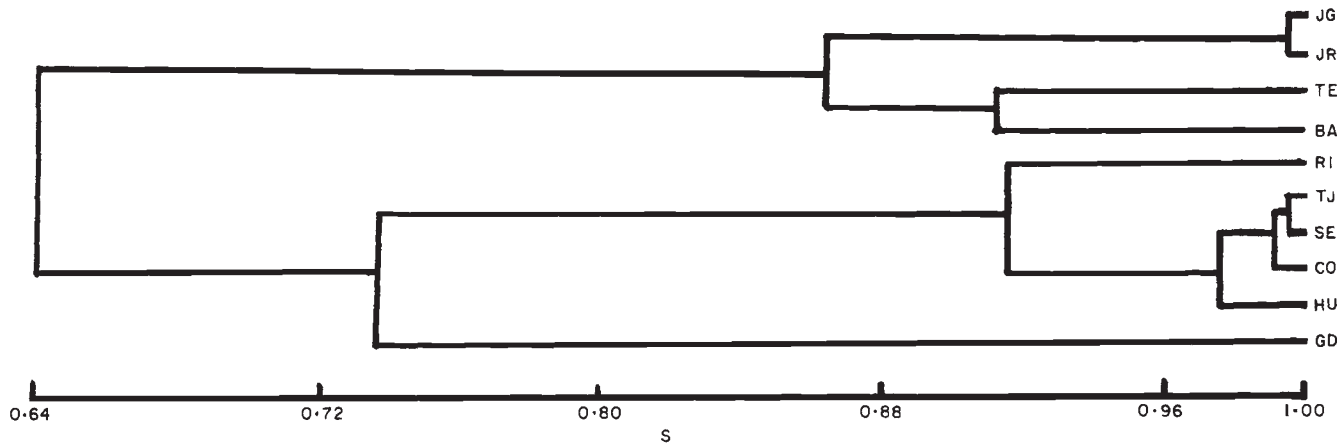

Fig. 2 UPGMA dendrogram of 10 populations of *Darlingtonea kentuckensis* based on Rogers' Coefficient of Genetic Similarity (*S*). Abbreviations as in Fig. 1b.

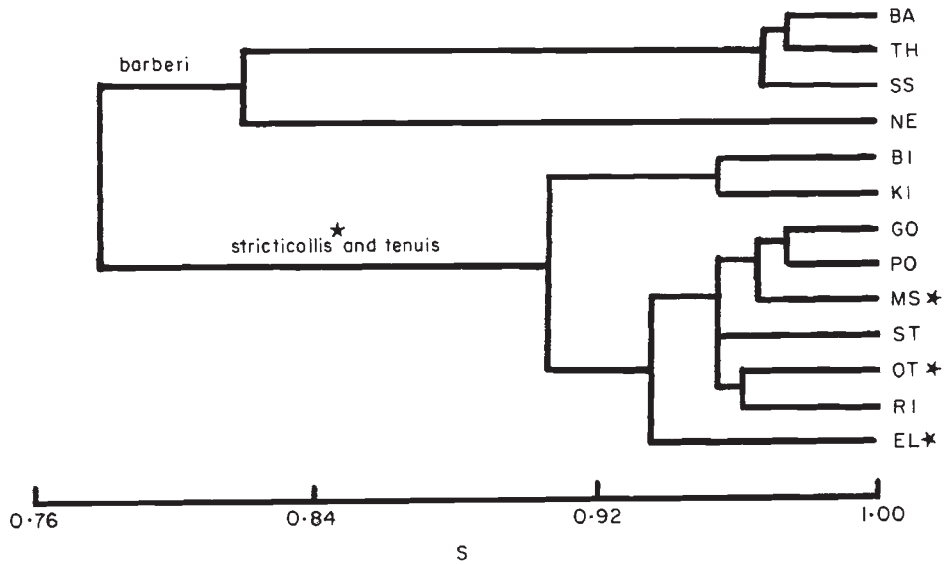

Fig. 3 UPGMA dendrogram of 13 populations of three *Pseudanophthalmus* spp. based on Rogers' Coefficient of Genetic Similarity (*S*). Abbreviations as in Fig. 1c.

and KI populations, near Corydon, IN and south of Indian Creek (Fig. 1c), have the 'D' electromorph at high frequencies, whereas the 'B' electromorph is prevalent in the four populations which lie to the north on the other side of Indian Creek (Table 2). The KI population is 5 km east of the BI population on the opposite side of Little Indian Creek (Fig. 1c) and is electrophoretically distinguished from all populations by the unique 'E' electromorph of PGI which occurs at significant frequency (Table 2). The differences among these *P. tenuis* populations suggest some type of dispersal barrier but it is not immediately obvious what that barrier might be. Neither Indian Creek nor its tributary Little Indian Creek meet the criteria for a fluvial barrier described by Barr (1985), but such base level master streams [Indian Creek flows at about or slightly below the level of the main stream in Binkley's Cave (site BI), and a number of subterranean streams empty into it as springs] may exert more control over cave trechine dispersal than has been previously suspected. There is no apparent stratigraphic barrier, and the distance between the southern and northern populations is not great (35–50 km). By contrast, although morphologically distinct (Barr, 1985), *P. stricticollis* and *P. tenuis* are not electrophoretically distinguishable from each other with the present data (Fig. 3).

Unlike *D. kentuckensis*, much of the genetic variation in the *Pseudanophthalmus* spp. occurs within

populations ($H = 0.094$; Table 4). Qualitatively, differentiation among *P. barberi* ($F_{ST} = 0.239$; Table 3) and among *P. tenuis* ($F_{ST} = 0.179$) populations can be described as great. Differentiation among the three *P. stricticollis* populations ($F_{ST} = 0.074$; Table 3) is slight to moderate.

Gene flow

Estimates of gene flow are lowest for *D. kentuckensis* with $N_m = 0.010$. Trexler (1988) suggests that values of $N_m < 0.5$ will cause gene pools to behave independently with regard to nearly neutral alleles under any gene flow model. The estimates of gene flow obtained for *P. barberi* ($N_m = 0.796$), *P. stricticollis* ($N_m = 3.13$) and *P. tenuis* ($N_m = 1.15$) are much larger, falling in the range of migration rates (i.e. $N_m \geq 1.0$) likely to stem population differentiation (see Trexler, 1988). Admittedly the geographical ranges of each of the *Pseudanophthalmus* spp. are less than that of the *D. kentuckensis* populations sampled.

The lack of gene flow among *D. kentuckensis* populations also appears to be manifested in the distribution of single locus coefficients of Rogers' *S* for the nine variable loci (Fig. 4a). The distribution is highly U-shaped with approximately 90 per cent of the coefficients having values of zero (complete dissimilarity) or one (genetic identity). The distribution of single locus *S* values among the 13 populations of the three related *Pseudanophthalmus* spp. is much less U-shaped (Fig. 4b). Only approximately 50 per cent of the comparisons assume values of zero or one, with the remaining comparisons spread over a wide range of intermediate values.

The extent of gene flow reduction in *D. kentuckensis* is further evidenced by comparing the data for it with data for its prey species, the cave cricket *Hadenoecus cumberlandicus*, over approximately the same geographical range. Electrophoretic data for eight populations of *H. cumberlandicus* (Caccone, 1985; Caccone & Sbordoni, 1987) yield lower estimates of differentiation ($F_{ST} = 0.46$) and higher estimates of gene flow ($N_m = 0.29$) than those we have observed for *D. kentuckensis*. Using Slatkin's (1981) graphical technique (Fig. 5), *H. cumberlandicus* can be described qualitatively as a species with intermediate gene flow levels as opposed to the low gene flow level in *D. kentuckensis*. *Hadenoecus cumberlandicus* is an obligatory trogloxene and, unlike *D. kentuckensis*, leaves the cave periodically to feed on the surface (Hubbell & Norton, 1978). Thus, the higher gene flow estimates for *H. cumberlandicus* may reflect higher present migration due to limited surface dispersal.

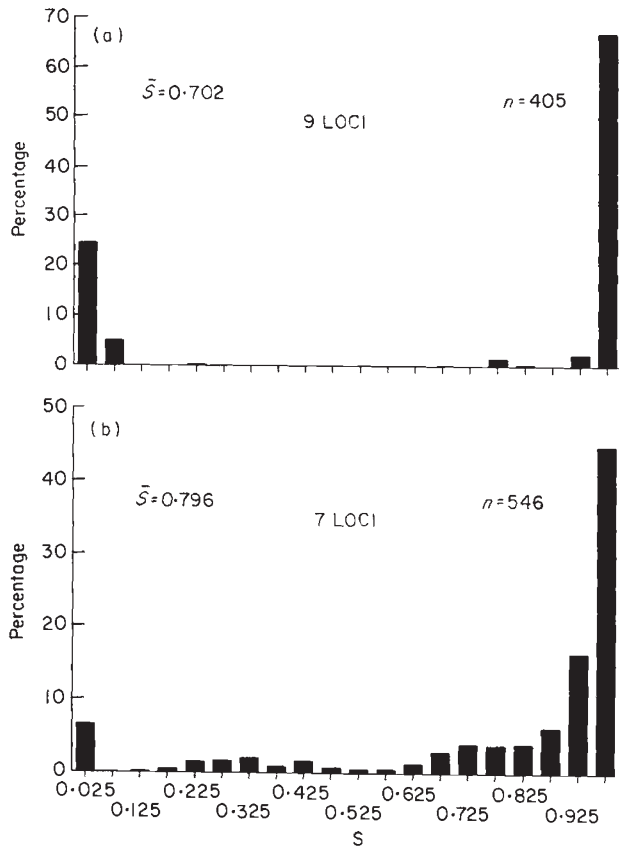

Fig. 4 (a) Frequency distribution of single locus values of Rogers' Coefficient of Genetic Similarity (*S*) for nine variable enzymatic loci in 10 populations of *Darlingtonea kentuckensis*. 4(b) Frequency distribution of single locus values of Rogers' Coefficient of Genetic Similarity (*S*) for seven variable enzymatic loci in 13 populations of three *Pseudanophthalmus* spp.

Discussion

The extremely low estimates of gene flow for the widely distributed polytypic troglobite *D. kentuckensis* indicate that the current lack of migration between geographical segments of the species is a condition which has persisted for some time. Much genetic differentiation in *D. kentuckensis* corresponds with major surface drainage systems in MP-II, supporting the view that these rivers are barriers to gene flow between terrestrial troglobitic populations (Barr, 1985). However, the greatest genetic differentiation occurs between the four northern and six southern populations (see Figs 1b and 2) over a region in which no river or other obvious geological barrier to gene flow is present. Thus, additional geological and/or historical factors may influence the genetic structure of *D. kentuckensis* populations.

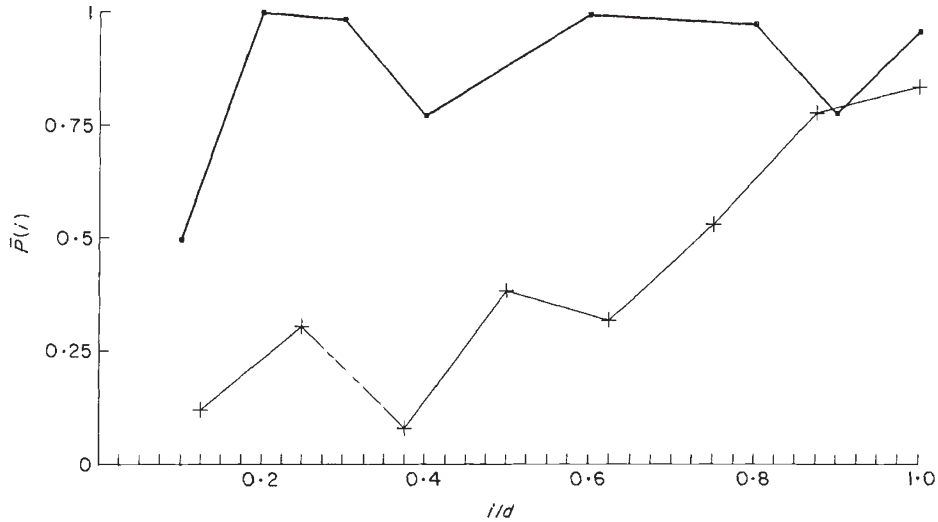

Fig. 5 Conditional allele frequencies (p_i) as a function of their incidence (i/d) in (—■—) *Darlingtonea kentuckensis* and in its prey species (—┼—) *Hadenoecus cumberlandicus*. (i) the number of demes in which an allele occurs; (d) the total number of demes sampled. Data for *D. kentuckensis* are from this study and data for *H. cumberlandicus* are from Caccone (1985). The pattern for *H. cumberlandicus* suggests intermediate levels of gene flow, whereas that for *D. kentuckensis* suggests low levels of gene flow among populations.

In MP-I the Ohio River is the boundary between *P. barberi* in the south and *P. tenuis* to the north. One scenario (Barr, 1985) postulates that these two species have arisen from a common ancestor whose range was divided by the entrenchment of the Ohio River during the early Pleistocene. Biochemical differentiation among *P. tenuis* populations raises the possibility that even smaller, youthful streams such as Indian Creek may disrupt gene flow and produce significant genetic divergence among local populations.

Note, genetic differentiation among the 13 *Pseudanophthalmus* populations is less than that among the 10 *D. kentuckensis* populations (see Fig. 4), despite the fact that the former include three distinct species. Geological differences between MP-I and MP-II may explain differences in genetic patterns of trechines between the two regions. MP-I is less dissected and has larger 'fragments' than MP-II (Barr, 1985). The *Pseudanophthalmus* populations show less differentiation and high within-population variability ($H = 0.094$) compared with *D. kentuckensis*. A more direct comparison can be had between the trechine *Neaphaenops tellkampfi* in MP-I and *D. kentuckensis*. *Neaphaenops tellkampfi* is probably the oldest troglobitic trechine in MP-I and, like *D. kentuckensis*, is a cricket egg predator (Barr, 1979). Furthermore, it has the largest geographical range of any troglobitic trechine and is differentiated into four morphological subspecies over its range (Barr, 1979). Patterns of differentiation ($F_{ST} = 0.559$) and variability ($H = 0.094$) among and within *N. tellkampfi* populations (Kane & Brunner, 1986) are more similar to those of the 13 *Pseudanophthalmus* populations than to *D. kentuckensis*, which suggests the common effect of the MP-I geology.

Despite the observed differences in the degree of genetic differentiation between species of MP-I and MP-II, the troglobitic trechines examined in this study are more differentiated than are some surface-dwelling carabids of the same region. Liebherr (1988) conducted an electrophoretic study of five carabid species of the genera *Agonum* and *Platynus* in the mid-Atlantic and southeastern United States. F_{ST} values ranged from 0.003 to 0.27 even though the populations sampled ranged over distances of 400–4100 km. For 17 populations of *A. decorum* (a winged species) collected over a 4100 km distance, F_{ST} was 0.08 (Liebherr, 1988). Thus, genetic differentiation among local populations of these surface-dwelling carabid species is generally less than what we observe among troglobitic trechine populations over much shorter geographical distances.

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