GEOGRAPHIC VARIATION IN THE CAVE BEETLE *NEAPHAENOPS TELLKAMPFI* (COLEOPTERA: CARABIDAE)

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INTRODUCTION

More than 200 species of cave limited (i.e., troglobitic) trechine carabid beetles are known from caves of the eastern United States (Barr, 1979b, 1981). These species are generally considered to be derived from ancestral surface species which were widespread during the cold, moist climates associated with glacial maxima (Barr, 1968). Subsequent warming and drying of these regions, as glaciers retreated, led ultimately to the extirpation of surface populations, with only some of the cave limited stocks surviving. Available evidence suggests that for trechines cave isolation is irreversible (Barr, 1968, 1979a). Therefore, geographic spread of and gene flow in troglobitic trechines will be restricted to subterranean routes (Barr, 1968). The interconnectivity of caves and the presence of geological barriers (e.g., noncavernous strata and large rivers) become important factors in determining the geographic extent of and degrees of gene flow within these troglobitic taxa.

In extensive and highly continuous limestone cave systems, such as those of the Mississippian plateaus, interpretation of evolutionary relationships between closely similar taxa becomes especially complicated (Barr, 1979b). One question which arises is whether such taxa represent multiple isolations of a common surface dwelling ancestor or are the product of more recent divergence in a common troglobitic ancestor. Even when the latter scenario appears to be the case, divergence may only involve subtle, although generally consistent, differences in minor morphological characters. Thus, inferences about such factors as the amount of gene flow, if

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any, still occurring among the taxa, the relative degree of differentiation between the various taxa, and the manner in which the present geographic pattern has been produced may be strengthened by the availability of genetic data such as those obtained through gel electrophoresis (Barr, 1979b; Turanchik and Kane, 1979).

As Barr (1979b) has indicated, the large geographic distribution and abundance of *Neaphaenops tellkampfi* populations present an excellent opportunity to assess the extent of gene flow between local populations of a troglobitic trechine using both morphological and electrophoretic data. Among the many species of troglobitic trechine carabid beetles in the United States, *Neaphaenops tellkampfi* is noteworthy for having the most extensive geographic range and being one of the most abundant species of the group (Barr, 1979b, 1981). The species is distributed (Fig. 1) from just south of the Ohio River in the north to its southern limit near the Tennessee border, in the highly cavernous Mississippian limestones of the Pennyroyal Plateau in west central Kentucky (Barr, 1979b). The western extent of its range is delimited by the noncavernous Big Clifty sandstone, and the eastern and southeastern limits of the range correspond roughly with the contact with the Salem and Warsaw limestones (Barr, 1979b).

Neaphaenops tellkampfi, like other cave trechines, is an important predator in terrestrial cave communities (Barr and Kuehne, 1971; Kane and Poulson, 1976). Unlike other troglobitic trechines in the Pennyroyal Plateau, however, *N. tellkampfi* has evolved specialized behaviors which allow it to prey on the eggs and early instar nymphs of the common cave "cricket" *Hadenoecus subterraneus* (Orthoptera: Rhaphidophoridae), resources which are energy rich and seasonally abundant (Kane and Poulson, 1976; Hubbell and Norton, 1978). This predator-prey interaction has evolved to the extent that no *N. tellkampfi* populations occur outside the range of *H. subterraneus* (Hubbell and Norton, 1978). In fact, Barr (1979b) has suggested that at least part of the eastern limits of the *N. tellkampfi* range may be determined by the absence of *H. subterraneus* further east, rather than to the presence of any extrinsic geological barrier.

Using morphological and geological criteria, Barr (1979b) has recognized four subspecies of *N. tellkampfi.* The nominate subspecies, *N. t. tellkampfi,* on which most of the ecological studies dis-

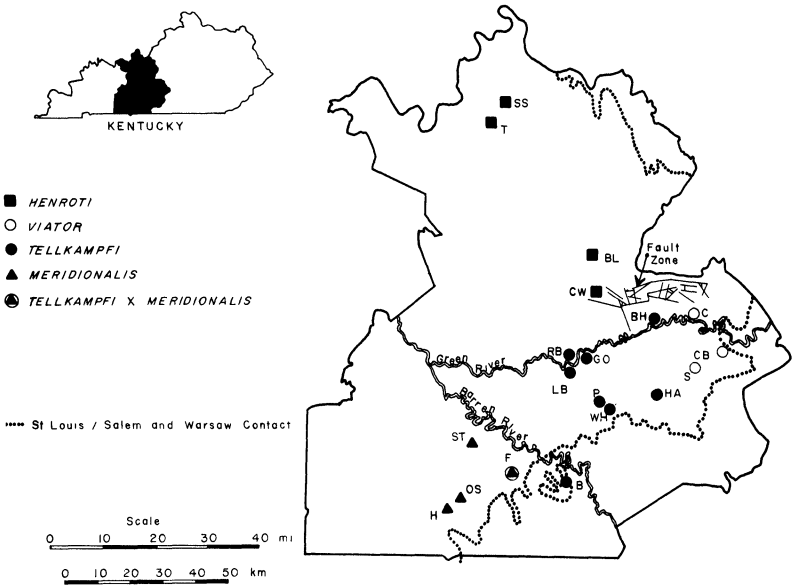

Figure 1. Map of west central Kentucky showing locations sampled for *Neaphaenops tellkampfi* in this study. Taxonomic designations of populations at these sites (after Barr, 1979b) are as follows: *N. t. henroti:* BL; CW; SS; T; *N. t. meridionalis:* H; OS; ST; *N. t. tellkampfi:* B; BH; GO; HA; LB; P; RB; WH; *N. t. viator:* C; CB; S; *N. t. meridionalis* × *N. t. tellkampfi* hybrid: F.

cussed previously have been done, is distributed in the central portion of the range to include the caves of Mammoth Cave National Park. *Neaphaenops t. meridionalis,* the southern subspecies, is limited to the north by the noncavernous sandstones near the Barren River. However, two populations are known in the southeastern part of the range which are morphologically intermediate between nominate *tellkampfi* and *meridionalis* for six of nine diagnostic characters, suggesting a narrow zone of hybridization between the two subspecies. Barr (1979b) points out, however, that despite the limited gene flow, *meridionalis* is morphologically the most distinct of the four subspecies. Morphological evidence (Barr, 1979b) suggests a broad zone of hybridization between nominate *tellkampfi* and the eastern subspecies *N. t. viator,* with gradual intergradation between the two subspecies over approximately an

eight km. distance. The eastern extent of the *viator* range is delimited by the contact of the St. Louis/Salem and Warsaw limestones and, perhaps more directly, by the absence of *H. subterraneus* further east (Barr, 1979b). As is the case with nominate *tellkampfi,* populations of *viator* are known from caves on both the north and south sides of the Green River. The northern limits of the *viator* range are set in large part by a sandstone ridge and extensive fault zone across Hart County. This geological feature also appears to be a complete barrier to gene flow between the northern subspecies *N. t. henroti* and either nominate *tellkampfi* or *viator* to the south (Fig. 1) (Barr, 1979b). Despite the absence of any known hybrid populations, *tellkampfi* and *henroti* are the most similar subspecies morphologically, and *henroti* also shows a large degree of morphological affinity with *viator* as well (Barr, 1979b).

Previous studies using gel electrophoresis (Giuseffi et al., 1978; Turanchik and Kane, 1979) have shown that genetic variability in local populations of *N. t. tellkampfi* approach those observed in similar surface dwelling invertebrates. These results, coupled with similar subsequent findings in other species (e.g., Dickson et al., 1979), suggest that cave adaptation does not necessarily result in a reduction in genetic variation. Further, genetic similarity values (I) (Nei, 1972) among eight local populations of nominate *tellkampfi* fall in the range (i.e., 0.90–1.00 (Turanchik and Kane, 1979)) commonly reported for populations of continuously distributed surface dwelling species. These results substantiate the contention that continuous limestone expanses can act as underground dispersal highways for cave limited species (Barr, 1968).

The purpose of the present study was to examine electrophoretically several local populations of each of the other three subspecies of *N. tellkampfi.* We were interested in determining how infrasubspecific variation in these subspecies compared with that of nominate *tellkampfi.* Further, we wished to use these electrophoretic data to quantitatively assess relationships among subspecies and also to gain some insight to how the present distributional pattern of the species has been produced. In these regards, Barr's (1979b) morphological and biogeographic work provides a model against which the electrophoretic data can be examined.

METHODS

Electrophoretic data gathered from a total of 18 populations (Fig. 1) of *Neaphaenops tellkampfi* were analyzed in this study. All of the electrophoretic data for ten of these populations were gathered during the course of the present study, between 1980 and 1983. These ten populations include three each of *N. t. henroti* (BL, CW and T/SS; Fig. 1), *N. t. meridionalis* (H, OS and ST; Fig. 1) and *N. t. viator* (C, CB and S; Fig. 1) as recognized by Barr (1979b). The tenth population (F; Fig. 1) is a purported *meridionalis* × *tellkampfi* hybrid on morphological grounds (Barr, 1979b). Most, but not all, of the electrophoretic data on the eight populations of *N. t. tellkampfi* (B, BH, GO, HA, LB, P, RB and WH; Fig. 1) were collected in 1977–78 and reported by Turanchik and Kane (1979). Modifications of and additions to the nominate *tellkampfi* data set will be discussed in appropriate sections below. All 18 of the populations sampled, with the exception of the SS and T sites of *henroti,* represent a single cave location. During the course of the study permission to sample the SS site was rescinded before a sample adequate for complete electrophoretic survey could be obtained. Subsequently the nearby T site was located but it harbored a much smaller *henroti* population and failed to yield a large enough sample to obtain data on all electrophoretic loci. Pooling of the data from the two sites, which appears to be justified by their geographic proximity, did produce a complete set of electrophoretic data.

Beetles were maintained alive at 5°C or frozen at -80°C prior to electrophoresis. All electrophoresis was conducted on vertical polyacrylamide slab gels using an Ortec Model 4200 Electrophoresis System or a Hoefer Scientific SE600 System. Sample preparation and run procedures used in this study were similar to those discussed by Giuseffi et al. (1978) and Turanchik and Kane (1979). Each animal provided enough homogenate for two assays.

Six enzyme systems provided a total of seven consistently scorable loci. These included: alkaline phosphatase (ALP) (1); esterase (EST) (1); malate dehydrogenase (MDH) (2); phosphoglucomutase (PGM) (1); phosphoglucose isomerase (PGI) (1); and, xanthine dehydrogenase (XDH) (1). In addition a general protein (GP) stain revealed two sets of consistently scorable bands which are also

included in the data. The more complete data of this study suggested interpretational changes at two loci from those reported by Turanchik and Kane (1979). The present data show that the ALP bands are properly interpreted as a single variable locus rather than as two separate loci. Also, we have chosen a more conservative interpretation of the XDH data. Electrophoretic analysis of XDH in *N. tellkampfi* populations produces a single band per beetle with slight differences in mobility between some individuals. Initially these data appeared to be consistent with data reported by Singh et al. (1976) for a variable XDH locus in *Drosophila pseudoobscura.* However, application of additional techniques which Singh et al. (1976) used to reveal multiple bands in *D. pseudoobscura* heterozygotes, failed to reveal any multiple banded *N. tellkampfi* individuals at the XDH locus. More recently, Finnerty and Johnson (1979) have shown that data such as these may be the result of post-translational modification of an enzyme encoded by a monomorphic locus. We have chosen this interpretation of the XDH locus in the present study. PGM was not assayed in previous studies of *N. tellkampfi* (Giuseffi et al., 1978; Turanchik and Kane, 1979) and therefore populations of *N. t. tellkampfi* were re-collected and surveyed for this enzyme. The majority of the data analysis was accomplished using a Fortran 77 version of the BIOSYS-1 program developed by Swofford and Selander (1981).

Results

Of the nine putative genetic loci examined in this study, five were polymorphic and the remaining four were monomorphic with the same variant fixed in all populations of the four taxa (Table 1). Genetic variability in *N. tellkampfi* populations has been estimated as the proportion of polymorphic loci per population (P) and the average frequency of heterozygous loci per individual (H) (Table 2). The average *N. tellkampfi* population is polymorphic at approximately 30% of its loci and the average individual in such a population is heterozygous at 9.4% of its loci (Table 2). These values are somewhat lower than those reported previously by Turanchik and Kane (1979) as a result of the addition of another invariant locus (PGM) and the more conservative interpretation of the XDH locus. However, these values of P and H still approach values typically reported for many surface invertebrates (Selander, 1976). Therefore,

Table 1. Gene frequencies for 18 populations of *Neaphaenops tellkampfi*. N = Sample size.

		Cave																	
Locus		CW	BL	T/SS	H	OS	ST	F	B	BH	GO	HA	LB	P	RB	WH	C	CB	S
1. Monomorphic loci with the same variant fixed in all populations—4 loci (GP-2; MDH-1; PGM; XDH)																			
2. Loci coding for monomorphic and/or polymorphic proteins.																			
ALP	N	27	29	10	25	42	11	7	15	20	14	10	11	13	16	10	21	9	22
	a	0.41	0.43	0.45	0.48	0.39	0.55	0.57	0.67	0.78	0.54	0.30	0.46	0.58	0.59	0.70	0.52	0.33	0.61
	b	0.59	0.57	0.55	0.52	0.61	0.45	0.43	0.33	0.22	0.46	0.70	0.54	0.42	0.41	0.30	0.48	0.67	0.39
EST	N	20	27	11	30	25	12	8	21	22	40	31	29	33	21	15	21	9	22
	a	0.00	0.00	0.00	1.00	1.00	1.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
	c	1.00	1.00	1.00	0.00	0.00	0.00	0.25	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
GP-1	N	30	29	18	28	23	15	17	8	13	6	13	22	10	19	15	23	13	17
	a	0.08	0.22	0.11	0.29	0.33	0.00	0.29	0.06	0.08	0.00	0.23	0.00	0.10	0.08	0.07	0.02	0.31	0.26
	b	0.92	0.78	0.89	0.71	0.67	1.00	0.71	0.94	0.92	1.00	0.77	1.00	0.90	0.92	0.93	0.98	0.69	0.74
MDH-2	N	33	36	10	59	33	11	18	23	28	26	20	24	29	37	20	22	18	30
	a	0.91	0.93	1.00	0.89	0.95	1.00	1.00	0.94	0.79	0.83	0.90	0.96	0.91	0.77	0.93	0.93	1.00	0.97
	b	0.09	0.07	0.00	0.11	0.05	0.00	0.00	0.06	0.21	0.17	0.10	0.04	0.09	0.23	0.07	0.07	0.00	0.03
PGI	N	38	43	9	29	30	13	18	20	22	42	30	24	20	24	22	30	23	27
	a	0.00	0.00	0.00	1.00	1.00	1.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
	c	1.00	1.00	1.00	0.00	0.00	0.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00

these data continue to support the contention that cave isolation does not necessarily result in a permanent reduction in genetic variability for a species (Barr, 1968; Giuseffi et al., 1978; Dickson et al., 1979; Turanchik and Kane, 1979).

Estimates of P and H by subspecies (Table 2) indicate no differences in genetic variability between the four taxa. Of the five polymorphic loci examined, two, EST and PGI, are diagnostic of subspecific differentiation (Table 1). Three variants have been detected at each of these loci, with all *meridionalis* populations fixed for a slow migrating electromorph at both loci, all *viator* populations fixed for electromorphs of intermediate mobility at each locus, and all *henroti* and nominate *tellkampfi* populations being fixed for the fast migrating electromorphs at both loci. The only local population that is polymorphic at these two loci is population F (Fig. 1 and Table 1). This population, which is morphologically intermediate between *meridionalis* and *tellkampfi* and a purported hybrid of the two subspecies (Barr, 1979b), contains both the slow and fast electromorphs at both the EST and PGI loci. The fact that these electromorphs are alternatively fixed in *meridionalis* and *tellkampfi* populations respectively provides biochemical evidence of the hybrid nature of this population. By contrast, all three of the *viator* populations are fixed for the intermediate mobility electromorphs at both the EST and PGI loci even though two of these populations, C and S, lie in the zone of morphological intergradation between *tellkampfi* and *viator* (Barr, 1979).

Rogers' (1972) estimate of genetic similarity (S) was used for pairwise comparisons of the 18 populations (Table 3). Rogers' distance values were used in a UPGMA clustering procedure to produce a biochemical dendrogram (Fig. 2). Infrasubspecific genetic identities are all greater than 0.90. This includes some populations, such as the C population of *viator,* separated from other populations of the same subspecies by shallow rivers such as the Green River. This finding is consistent with earlier work (Turanchik and Kane, 1979) on populations BH, RB and B of nominate *tellkampfi* and with findings on at least one other cave limited species in the same area (Laing et al., 1976), and serves to reconfirm the fact that rivers per se are not necessarily dispersal barriers for cave limited forms. Genetic differentiation between subspecies is substantial in some cases (Fig. 2). *Neaphaenops t. meridionalis* and *N. t. viator*

Table 2. Genetic variability in four subspecies of *Neaphaenops tellkampfi*. P = average proportion of polymorphic loci per population; H = average proportion of heterozygous loci per individual.

Subspecies	Site	P	H OBS.	H EXP	Avg. Alleles/ Locus
henroti	CW	0.333	0.088	0.091	1.145
	BL	0.333	0.117	0.110	1.182
	T/SS	0.222	0.091	0.081	1.187
	AVG	0.296	0.099	0.094	1.171
meridionalis	H	0.333	0.133	0.124	1.216
	OS	0.333	0.119	0.113	1.201
	ST	0.111	0.040	0.058	1.109
	AVG	0.259	0.097	0.098	1.175
tellkampfi	B	0.333	0.058	0.079	1.116
	BH	0.333	0.077	0.094	1.132
	GO	0.222	0.088	0.086	1.153
	HA	0.333	0.107	0.111	1.166
	LB	0.222	0.070	0.067	1.119
	P	0.333	0.076	0.092	1.152
	RB	0.333	0.073	0.112	1.184
	WH	0.333	0.076	0.079	1.114
	AVG	0.305	0.078	0.090	1.142
viator	C	0.333	0.093	0.075	1.132
	CB	0.222	0.101	0.102	1.171
	S	0.333	0.101	0.104	1.177
	AVG	0.296	0.098	0.094	1.160
mer. × *tell.* hybrid	F	0.444	0.192	0.167	1.265
			Neaphaenops tellkampfi		
	AVG	0.302	0.094	0.097	1.162
	OVERALL	0.556			

show levels of similarity to each other and to the other two subspecies in the range of 0.69–0.78 (Fig. 2). Genetic similarity between *henroti* and nominate *tellkampfi* ($S > 0.96$; Table 3) is as great as similarity values among local populations within a subspecies. Although these two subspecies are the most similar of the four

Table 3. Rogers' (1972) coefficients of genetic similarity (S) for comparisons of four subspecies of *Neaphaenops tellkampfi*. Values shown are averages of pairwise comparisons of appropriate populations. Values in parentheses are the ranges of similarity values appropriate to each comparison.

		Subspecies			
Subspecies	No. of Pops.	*N. t. h.*	*N. t. m.*	*N. t. t.*	*N. t. v.*
henroti	3	0.975 (0.971–0.978)			
meridionalis	3	0.748 (0.732–0.766)	0.956 (0.942–0.983)		
tellkampfi	8	0.963 (0.928–0.982)	0.730 (0.689–0.763)	0.963 (0.917–0.995)	
viator	3	0.741 (0.714–0.758)	0.740 (0.727–0.766)	0.737 (0.713–0.765)	0.963 (0.945–0.984)
mer. × *tell.* hybrid	1	0.878 (0.863–0.886)	0.833 (0.828–0.836)	0.865 (0.844–0.883)	0.770 (0.750–0.781)

morphologically, this large a genetic similarity is somewhat unexpected given the presence of the Hart Co. Ridge, an apparent geological barrier between these two subspecies.

Genetic differentiation within and between subspecies was examined using F-statistics (Wright, 1978) and a Chi-square contingency analysis of heterogeneity (Workman and Niswander, 1970). Allozyme phenotype frequencies for the 18 populations were used to calculate genetic differentiation (i.e., F-statistics) in a hierarchichal manner (Wright, 1978). The two hierarchical levels are subspecies within species and local populations within subspecies. Since the hybrid F population could not be unequivocally assigned to either *tellkampfi* or *meridionalis,* it was considered as a fifth "subspecies" at that level of the hierarchy. Three loci (ALP; GPT-1; MDH-2) are variable in some or all local populations of each subspecies. Significant heterogeneity in gene frequencies (Chi-square) was observed among *N. t. tellkampfi* populations at the ALP and MDH-2 loci but not at the GPT-1 locus (Table 4). Significant heterogeneity in gene frequencies at the GPT-1 locus was observed among local populations of *viator* and among local populations of *meridionalis,* but no differentiation was observed among local populations of either subspecies at the ALP or MDH-2 loci (Table 4). No heterogeneity in gene frequency was observed among *henroti* populations at any of

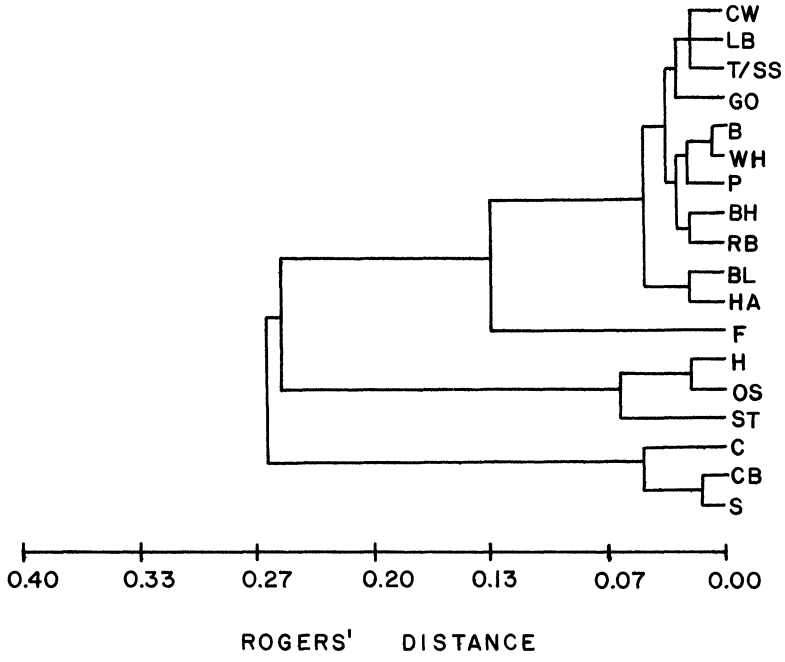

Figure 2. UPGMA dendrogram of 18 populations of *Neaphaenops tellkampfi*, generated from Rogers' genetic distance values for nine biochemical loci.

the three variable loci. The slightly greater differentiation observed among *tellkampfi* populations may be due to the fact that this subspecies has a somewhat larger geographic range than any of the other three subspecies, or simply to the fact that more populations (8) were examined for nominate *tellkampfi* than for any of the other three subspecies.

Whereas genetic differentiation between infrasubspecific populations is slight to moderate, differentiation between subspecies is very great (Table 5). At the level of subspecies, variation is observed at the EST and PGI loci in addition to the three loci discussed above. Significant heterogeneity in allele frequency between subspecies was observed at all five loci (Table 5) and overall genetic differentiation is very great (Fst = 0.528), with the EST and PGI loci essentially fixed for alternative alleles in three of the four subspecies.

Table 4. F-statistics and heterogeneity chi-square values for four subspecies of *N. tellkampfi.*

	F_{IT}	F_{IS}	F_{ST}	X^2
SUBSPECIES		ALP LOCUS		
henroti	-0.007	-0.013	0.006	0.510ns
meridionalis	0.098	0.083	0.016	1.690ns
tellkampfi	0.217	0.150	0.080	16.416*
viator	-0.183	-0.201	0.015	1.291ns
		GPT-1 LOCUS		
henroti	-0.166	-0.207	0.034	5.613ns
meridonalis	-0.004	-0.154	0.130	12.145***
tellkampfi	-0.083	-0.157	0.064	13.810ns
viator	0.193	0.105	0.098	12.465***
		MDH-2 LOCUS		
henroti	-0.007	-0.089	0.006	1.958ns
meridionalis	-0.055	-0.100	0.042	4.549ns
tellkampfi	0.209	0.176	0.041	17.605*
viator	-0.034	-0.058	0.022	2.539ns

ns = $P > 0.05$; * = $P < 0.05$; *** = $P > 0.005$

F_{IT} = correlation between uniting gametes relative to the gametes of the total population

F_{IS} = average correlation over subdivisions of uniting gametes relative to those of their own subdivision

F_{ST} = correlation of random gametes within subdivisions relative to gametes of the total population

Slatkin (1981) has proposed a method to estimate overall gene flow in natural populations in a qualitative manner from gene frequency data. Using computer simulation, Slatkin (1981) has demonstrated a dependence between gene flow and the conditional average frequency of an allele, $\bar{p}(i)$ where:

d = number of demes sampled

i = number of demes in which the allele occurs

$\bar{p}$ = average frequency of the alleles in those demes

Caccone (1985) used Slatkin's technique to assess gene flow in several species of cave animals, based on her own data for *H. subterraneus,* the data of Laing et al. (1976) for the scavenger beetle *Ptomaphagus hirtus* and Turanchik and Kane's (1979) data for the

Table 5. Hierarchichal F-statistics and heterogeneity chi-square analyis of allelic frequencies between subspecies of *Neaphaenops tellkampfi*

Locus	F_{CT}	F_{CS}	F_{ST}	X^2
ALP	0.022	0.023	-0.001	16.224**
EST	0.958	0.000	0.958	1564.120***
GPT-1	0.081	0.074	0.007	22.227**
MDH-2	0.044	0.035	0.009	17.900**
PGI	0.988	0.000	0.988	1841.171***
TOTAL	0.546	0.038	0.528	

** = $P < 0.01$; *** = $P < 0.005$

F_{CT} = correlation of random gametes in local populations relative to the gametes of the total population

F_{CS} = average correlation over subspecies of uniting gametes relative to those of their own subspecies

F_{ST} = correlation of random gametes within subspecies relative to gametes of the total population

subspecies *N. t. tellkampfi.* Thus, an analysis of gene flow in all four *N. tellkampfi* subspecies is appropriate since both *H. subterraneus* and *P. hirtus* are sympatric with *N. tellkampfi.* Further, the range of *H. subterraneus* examined by Caccone (1985) is more comparable to that of *N. tellkampfi* (s.l.) than simply to that of nominate *tellkampfi.*

The Slatkin analysis suggests that *N. tellkampfi* may be qualitatively described as a species in which gene flow level is low. Alleles with low incidence values (i/d) have high conditional frequencies ($\bar{p}$) (Fig. 3). Caccone (1985) showed that *P. hirtus* is also a species with low gene flow levels. By contrast, *H. subterraneus* is seen to be a species with intermediate gene flow levels (Caccone, 1985). As indicated earlier, the range of *H. subterraneus* is larger than and includes the entire range of *N. tellkampfi.* Unlike *N. tellkampfi* and *P. hirtus,* however, *H. subterraneus* is troglophilic (facultative cave dweller) and thus is capable of some dispersal on the surface in addition to the subterranean routes available to troglobites. Analysis of the eight nominate *tellkampfi* populations indicates a high level of gene flow within this subspecies (Fig. 3) despite some heterogeneity in gene frequencies among these populations (Table 4). The overall pattern of gene flow is generally consistent with the pattern of genetic differentiation obtained from the F-statistics.

Discussion

The patterns of variation described here for *N. tellkampfi* provide a basis for understanding some of the factors which cause genetic differentiation in cave limited species. Barr (1979b) suggested that three different patterns of gene flow were indicated by the morphological and geological data on the four subspecies. These include: (1) no gene flow (*henroti* with either *tellkampfi* or *viator*); (2) very limited gene flow (*meridionalis* with *tellkampfi*); and, (3) moderate gene flow (*tellkampfi* with *viator*). Initially the biochemical data seem to support only pattern (2) with population F clearly containing *meridionalis* × *tellkampfi* hybrids and with other *meridionalis* and *tellkampfi* populations examined in this study showing no biochemical evidence of hybridization. Thus, the morphological data (Barr, 1979b) and now the biochemical data suggest that hybridization is restricted to a very narrow geographic area.

The allozyme data directly support only part of pattern (1). The relatively large genetic distance between *henroti* and *viator* ($D = 0.289$) and the lack of any biochemical, as well as morphological (Barr, 1979b), evidence of hybridization support the assertion that the Hart Co. Ridge is acting as a complete barrier to gene flow between these two subspecies. The large genetic similarity between *henroti* and *tellkampfi* ($S > 0.96$) does not lend support to the conclusion that these two subspecies are also extrinsically isolated from each other. However, allozyme studies on the scavenger beetle *P. hirtus* (Laing et al., 1976) show that a population north of the Hart Co. Ridge has a genetic similarity (I) of approximately 0.75 with two populations south of the Ridge in caves GO and RB, which are also occupied by nominate *tellkampfi.* Further, the Hart Co. Ridge coincides with the southern range limit of *Orconectes inermis* (Decapoda: Astacidae) and the northern range limit of *O. pellucidus,* two species of troglobitic crayfish whose ranges are almost completely separate (Hobbs and Barr, 1972). Thus the evidence for the Hart Co. Ridge as a dispersal barrier is overwhelming.

The close genetic similarity between *henroti* and *tellkampfi* is consistent with Barr's (1979b) supposition that all four subspecies of *N. tellkampfi* are descended from a common ancestral stock that became isolated in caves in the southern portion of the present range. Barr argues that *henroti* was derived from a peripheral population of nominate *tellkampfi* which penetrated north of the Hart

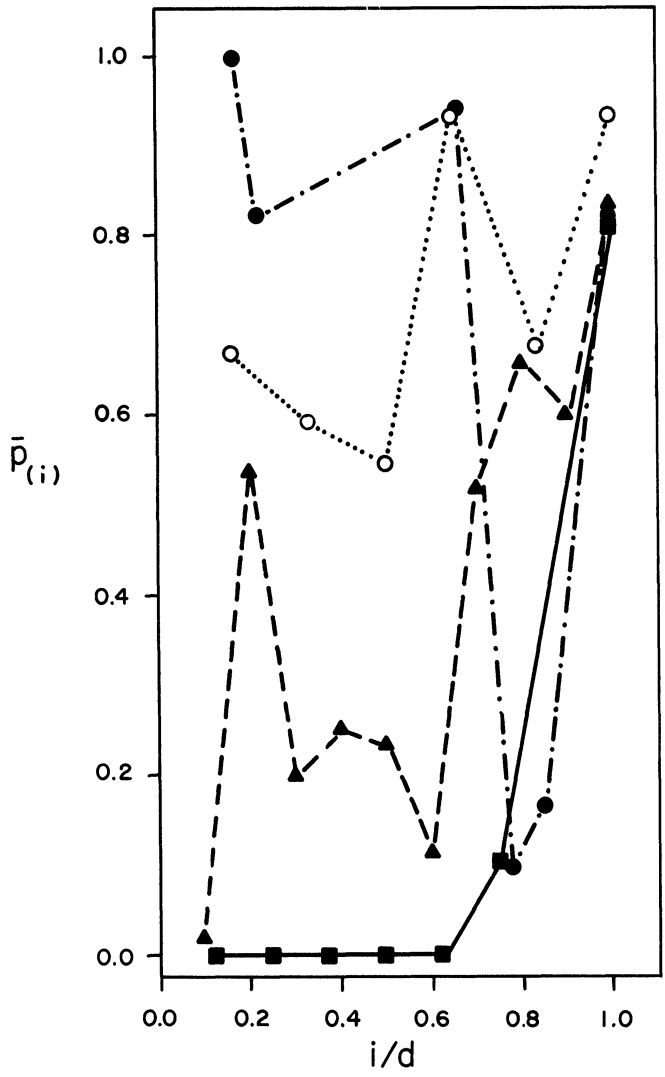

Figure 3. Conditional allele frequencies (p(i)) as a function of their incidence (i/d) in four taxa of cave-dwelling organisms. Three qualitative patterns of gene flow are inferred: low gene flow: *Neaphaenops tellkampfi* (filled circles) and *Ptomaphagus hirtus* [open circles (data from Laing et al., 1976)]; intermediate gene flow: *Hadenoecus subterraneus* [triangles (data from Caccone, 1985)]; and, high gene flow: *Neaphaenops tellkampfi tellkampfi* (squares).

Co. Ridge through some of the scattered cave systems known in the area. The close biochemical similarity of *henroti* and *tellkampfi* support this view over the alternative hypothesis that *henroti* represents a separate isolation of the surface dwelling ancestral species. Furthermore, Barr (1979b) notes that *henroti* has apparently not extended its range as far northward and westward as the geological evidence and the distribution of *Hadenoecus subterraneus* would suggest is possible. This observation, coupled with the evidence of high genetic similarity between *henroti* and *tellkampfi,* is supportive of a southern origin for *N. tellkampfi* with the range of *henroti* representing the most recent northward dispersal.

The allozyme data fail to demonstrate a broad zone of hybridization between *tellkampfi* and *viator* (pattern (3) above). Moreover, inclusion of additional information fails to explain the discrepancy between the biochemical distinctness of the two taxa, on the one hand, and the independent evidence for a broad zone of hybridization on the other. The lack of any geological barrier between *tellkampfi* and *viator* and the large degree of morphological intergradation between the two taxa (Barr, 1979b) give great support to the hypothesis of hybridization. Two of the *viator* populations examined in this study (i.e., C and S) lie within the zone of morphological intergradation, making the lack of biochemical hybridization even more puzzling.

Genetic differentiation in *N. tellkampfi* occurs primarily between subspecies, with high genetic similarity ($S > 0.90$) and only slight ($Fst < 0.05$) to moderate ($0.05 < Fst < 0.15$) genetic differentiation among infrasubspecific populations. Culver (1982) reanalyzed Laing et al.'s (1976) data on *P. hirtus* and found that the average between area Nei index for *P. hirtus* populations in the ranges of different *N. tellkampfi* subspecies was $I = 0.794$. The average I between *N. tellkampfi* subspecies from the present study is 0.791. Further, analysis based on conditional allele frequencies indicates that gene flow level in both species can be qualitatively described as low. Interestingly the two species differ greatly in their ecological and demographic characteristics (Kane, 1982) and a substantial amount of evidence suggests that *N. tellkampfi* has a longer evolutionary history of cave isolation than does *P. hirtus* (Laing et al., 1976; Barr, 1979b).

Caccone (1985) suggests that gene flow levels and degree of genetic differentiation in cave species may be influenced by their

degree of dependence on the cave environment. Troglobitic species such as *N. tellkampfi* and *P. hirtus,* which are restricted to subterranean routes of dispersal, might be expected to show lower gene flow levels and greater genetic differentiation than cave dwelling species which are still capable of some dispersal on the surface. Although its distribution is restricted to cave regions, *H. subterraneus* emerges from caves on warm humid evenings to feed. Thus, the intermediate levels of gene flow inferred for *H. subterraneus,* as opposed to low levels for the two troglobites, may result from limited surface dispersal. Morphological evidence (Hubbell and Norton, 1978) also suggests a lesser degree of geographic differentiation in *H. suberraneus* than in *N. tellkampfi* over approximately the same area. Morphological differences occur between southwestern populations of *H. subterraneus* (i.e., in the range of *N. t. meridionalis*) and those to the north. However, there is no significant morphological differentiation among the northern populations of *H. subterraneus* (Hubbell and Norton, 1978), whereas in the same region *N. tellkampfi* is morphologically differentiated into three distinct subspecies (i.e., *henroti, tellkampfi* and *viator*). Trogloxenes show less cave dependence than troglophiles. Such species often use caves only sporadically and only for shelter. Unfortunately no genetic data are available for trogloxenes which are partially or wholly sympatric with the species described above. Caccone (1985) does report genetic data for *Euhadenoecus puteanus,* a relative of *H. subterraneus,* which is a forest dweller and a sporadic trogloxene over a range from southern New York to Georgia. She finds relatively high levels of gene flow between five cave populations of *E. puteanus* which is at least consistent with the expectations for a trogloxene.

Although degree of cave dependence appears to play a major role in determining the degree of gene flow and genetic differentiation over the geographic range of cave dwelling species, ecological differences between species may also influence their genetic characteristics. *Neaphaenops tellkampfi* and *P. hirtus* are both troglobites and show similar biogeographic patterns of genetic differentiation. However, ecologically the two species are dissimilar. Whereas *N. tellkampfi* is a specialized predator which tends to establish large permanent populations (Kane and Ryan, 1983), *P. hirtus* is more opportunistic. Local populations may develop on small isolated patches of organic matter such as carrion or feces from reproduction by a few founders (Peck, 1973) and such populations are often

ephemeral. Thus, stochastic events may have a greater influence on the genetic characteristics of local *P. hirtus* populations than on those of *N. tellkampfi.* In fact, genetic variability in local *P. hirtus* populations (P = 0.154; H = 0.048 (Laing et al., 1976)) appears to be about half that of local *N. tellkampfi* populations (P = 0.302; H = 0.094). Further, the average Nei index between local *P. hirtus* populations in the range of *N. t. tellkampfi* is I = 0.874 (Culver, 1982), whereas the average I between local nominate *tellkampfi* populations is 0.981. Thus, if ecological differences influence genetic patterns of similarly cave dependent species, the effects appear to be manifested at the level of local populations.

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Summary

An understanding of patterns of geographic variation is important in interpreting evolutionary relationships between closely similar taxa and in inferring levels of gene flow between geographic populations. For obligate cave dwelling (i.e., troglobitic) species, dispersal and gene flow are restricted to subterranean routes. Thus, the interconnectivity of caves and the presence of geological barriers become important factors in determining the geographical distribution and the degree of gene flow among populations of troglobitic species.

Neaphaenops tellkampfi, a troglobitic trechine beetle, has the most extensive geographic range and is one of the most abundant of

the approximately 200 species of cave trechines in the eastern United States. Four morphological subspecies of *N. tellkampfi* have been described over its range in west central Kentucky. In the present study, electrophoretic data were collected on a total of 18 populations to include all four subspecies. These data support the hypothesis that *N. tellkampfi* has been derived from a single isolation of a surface dwelling ancestor. The present distribution has apparently resulted from a northward movement of the troglobitic stock through subterranean routes. Morphological (i.e., subspecific) differentiation appears to be directly related to the presence of partial and/or complete geological barriers to dispersal in certain portions of the range.

Comparison of genetic data on *N. tellkampfi* with those on other sympatric cave dwelling species suggests that level of gene flow and degree of genetic differentiation may be related to the degree of cave dependence of such species. Troglobites show lower levels of gene flow and greater genetic differentiation over their geographic ranges than do more facultative cave dwellers (e.g., troglophiles and trogloxenes) in which intermediate to high levels of gene flow have been reported. Ecological differences between species with similar degrees of cave dependence do not appear to produce differences in genetic patterns on a biogeographic scale. There is some evidence to suggest, however, that ecological differences between such species may affect genetic variability and genetic distance at the level of local populations.

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