

Stoping Shafts

Solution chimneys, vertical shafts, and waterfall shafts are all formed by the same mechanism — water draining vertically through the vadose zone along fractures of various sorts. In contrast, stoping shafts form primarily by the mechanical processes of rock failure and breakdown. The initiation point is a preexisting void at depth. Ceiling collapse drops rocks into the void, partially filling it, and creates new void where the rocks were removed. As this process continues, the void migrates upward as rock fall continues. Because the rubble created by the falling blocks takes up more volume than the solid rock from which it came, the initiating void space can be filled and the process brought to a halt. If, however, the initial void contains flowing water that dissolves the fallen blocks and removes them, the void volume grows and the upward stoping process continues until it breaks through at the land surface (Fig. 4).

Should this be stoping? Should all the other stopings be stopping? →

Because stoping shafts form primarily by mechanical rather than chemical processes, their development is not limited to carbonate rocks. The cavity that initiates the stopping process is usually formed by dissolution of carbonate rock, shafts are known that have stoped upward through sandstone, shale, and even overlying lava flows. Thus on the Coconino Plateau of Arizona there occur shafts and breccia pipes that have stoped through clastic rocks far above the cavities in the Redwall and Muav Limestones that were the initiation centers. It might be noted that mine tunnels can also serve as the initial void spaces so that mine subsidence is not an intrinsically different process.

MECHANISMS FOR THE DEVELOPMENT OF PITS AND SHAFTS

The chemistry involved when carbonate rocks are dissolved to form solution chimneys, vertical shafts, and waterfall shafts is not intrinsically different from the chemistry

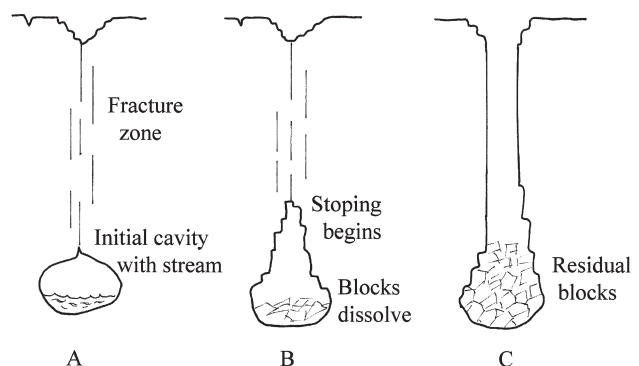
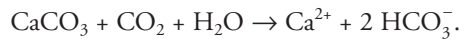


FIGURE 4 Sketches showing the evolution of a stoping shaft. (A) Fracture system and preexisting large chamber, (B) beginning of upward stoping process where fallen blocks are removed by dissolution in the large chamber, and (C) the shaft after it has broken through to the land surface. Incomplete dissolution of the fallen blocks may result in a completely obscured original chamber.

involved in the formation of horizontal caves. It may be summarized by a single reaction



However, there is a significant difference in the approach of the reaction to chemical equilibrium. Much of the dissolution of horizontal caves takes place in water that is close to saturation with calcium carbonate. Close to equilibrium, reaction rates are slow and the dissolitional enlargement of cave passages takes a long time. Once the vertical fractures have become enlarged even to a few millimeters, water drains through them very quickly. As a result, the fresh, unsaturated water that enters from the land surface or from the edge of a caprock descends through the vadose zone much more rapidly than the time required for chemical reactions to come to equilibrium. The result is that most shaft waters are highly undersaturated and are dissolving limestone at the fastest rate possible. A vertical shaft, a few meters in diameter, can form in a few thousand years.

Further Reading

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Population Structure

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Population structure is an important guideline to understanding the evolution of cave-dwelling animals, because it represents the outcome of their history and adaptation as well as the groundwork for speciation in the cave environment. However, despite its potential value, population structure is still a poorly studied feature of cave life.

Population structure can be viewed from two different perspectives. Ecologists usually view the composition of a population according to age and sex of individuals, and population geneticists keep in mind the organization of genetic variation within and between populations, with special emphasis on their spatial arrangement. In this article we address the latter aspect.

The advent of molecular techniques, from allozyme electrophoresis to microsatellite DNA analysis, led to significant improvements in understanding the genetics of natural

populations. Particularly, the feasibility to estimate parameters such as the amount of genetic variation, gene flow, time since isolation, etc., provides important evidence to test alternative hypotheses on patterns of colonization and evolution of cave populations.

The most obvious cause of structuring in cave populations is habitat fragmentation. However, as we will discuss, habitat fragmentation may not include the unique factor of population structuring. Generally speaking, caves reflect the historical process of habitat fragmentation quite well. Cave regions in temperate countries have been subjected to profound and repeated changes in climate and vegetation, which are particularly well documented in the Pleistocene. Cold and dry phases corresponding to glacial periods have repeatedly led to changes from mesophyllous forest to mountain steppe. Consequently, previously widespread ancestral populations of several cave-dwelling species became confined to small refugial habitats, leaving relictual populations at different steps of isolation, depending upon the biological properties and/or the history (i.e., time) of the organisms involved. Extrinsic causes for fragmentation can also be detected in other circumstances such as the structure and history of drainage systems, tectonic changes, or lava flow events in volcanic cave areas. In many cases these phenomena can be dated to a reasonable extent.

Other evolutionary processes can also account for population structuring, especially where population structure is perceived at much smaller scales, even within a single cave. Double or multiple invasion of a cave habitat at different times by different populations of a given species represents a feasible mechanism. Alternatively, extrinsic factors such as habitat and resource patchiness may interact with the intrinsic biological properties of organisms, like fecundity, dispersal ability, and behavioral characters, leading to a population structure maintained by different forms of natural selection.

In the following article, a series of study cases will be discussed to underscore the role of different factors in shaping population structure in a limited sample of cave organisms that have been the object of appropriate population genetic studies.

ESTIMATING GENETIC STRUCTURE

The genetic parameters relevant to investigate population structure are effective population size (N_e), observed and expected heterozygosity (H_o and H_e , respectively), genetic distance (D), amount of structuring between subpopulations (F_{ST}), and gene flow ($N_e m$, where m is the migration rate).

Effective Population Size (N_e)

The number of individuals that effectively participate in producing the next generation is named effective population size. Generally, the effective size of a population is consid-

erably less than the census size. Evolutionary processes are greatly influenced by the size of populations.

Heterozygosity

Mean heterozygosity, calculated across a number of loci, is a valuable parameter used to estimate the degree of genetic variation within a population. Population structuring occurs when genotype frequencies deviate from Hardy-Weinberg expected proportions, or panmixia is unfulfilled. If inbreeding or selection occurs, then populations can be considered "structured" in some way.

Genetic Distance

When two populations are genetically isolated, both mutation and genetic drift lead to differentiation in the allele frequencies at selectively neutral loci. As the amount of time that two populations are separated increases, the difference in allele frequencies between them should also increase, until each population is completely fixed for separate alleles. Therefore, calculation of genetic distance (D) between two populations provides a relative estimate of the time elapsed since these populations have existed as a single panmictic unit. Small estimations of distance among completely isolated populations indicate that they have only been separated for a short period of time. Alternatively, in the absence of isolation, small values of genetic distance may indicate population structure (i.e., subpopulations in which there is random mating, but between which there is a reduced amount of gene flow).

F-Statistics

F-statistics, developed by Wright (1965), represent the basic method to measure the amount of subdivision in populations. F-statistics can be viewed as a measure of the correlation of alleles within individuals, and they are related to inbreeding coefficients. An inbreeding coefficient is really a measure of the nonrandom association of alleles within an individual. As such, F-statistics describe the amount of inbreeding-like effects within subpopulations, among subpopulations, and within the entire population. In particular, the F_{ST} index is an estimator of the amount of structuring of a population into subpopulations.

Migration

If there is no migration (gene flow) occurring between two populations or demes, eventually alternate alleles will become fixed and will reach 1. Alternatively, it has long been known that if migration, measured in terms of $N_e m$, is >1 (where N_e is the effective population size and m is the proportion of migrants per generation, or migration rate), the allele frequencies in the subpopulations remain homogenized

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AU: → (Wright, 1931). If, however, migration is present but $N_e m < 1$, an equilibrium based on the rate of mutation, migration, and genetic drift will be established.

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POPULATION STRUCTURE AT REGIONAL SCALE: TERRESTRIAL CAVERNICOLES

It is well known that troglobitic species (i.e., obligate cave dwellers) occupy very reduced distribution ranges, sometimes limited to a given karst region or even to a single cave system. Troglophilic species (i.e., species able to live and reproduce in the cave habitat as well as in surface habitats) comparatively show much wider distribution ranges.

Related troglobitic species within a genus are often geographically vicariant in different karst areas or cave systems. A huge number of taxa in terrestrial and aquatic organisms, such as carabid and leiodid beetles, spiders, pseudo-scorpions, millipedes, isopods, amphipods, etc., exemplify this situation, particularly in limestone areas of temperate regions. These vicariant species represent the outcome of a geographical speciation process that was initiated with the population genetic structuring of an ancestral troglphilic species at the regional scale.

As already outlined, the amount of population structure is strictly dependent on intrinsic factors, such as dispersal ability of the organism implied and extrinsic factors limiting gene flow between populations, such as geographic distance and the extent of ecological and geographical barriers to migration. Therefore we cannot automatically expect that different organisms experiencing similar evolutionary pathways show the same geographical pattern of population structure. Careful genetic analyses are required to reveal the occurrence of actual or potential gene exchange between populations. In turn these analyses represent a tool to disclose historical relationships between populations and to

test hypotheses on processes generating spatially structured population systems.

Potential or actual gene exchange between cave populations, and their resultant structuring at a regional scale, can be understood by illustrating study cases involving rhabdiphorid crickets and nesticid spiders living in Italy and the eastern United States. By means of allozyme polymorphisms, levels of gene flow between populations were evaluated for *Nesticus* cave spiders and cricket populations belonging to *Dolichopoda laetitia* from the Italian peninsula, and to *Euhadenoecus puteanus* and *E. fragilis* from the eastern United States. The enhanced degree of population fragmentation in these organisms is chiefly the result of a gradual reduction of gene flow between populations caused by bioclimactic changes that occurred in the Pleistocene. Hence, levels of gene flow ($N_e m$), as measured on the basis of the present patterns of genetic differentiation (Wright's F_{ST}), are supposed to reflect historical gene flow occurring between cave and surface populations living in a continuum of wet or mesophyllous woody environments.

The amount of genetic structuring was estimated for multiple combinations of populations at different geographic scales. F_{ST} and $N_e m$ values were calculated for different groups of populations by considering increasingly wider geographic windows. The size of the geographic window for the population sampling was progressively enlarged from a minimum average population distance of 12–14 km for Italian caves and 30–40 km for American caves, to a maximum average distance of larger than 200 km (Fig. 1). Moreover, in *D. laetitia*, estimates of F_{ST} and $N_e m$ have also been performed at smaller geographic windows by means of samples caught in sites 50–500 m apart. At the same geographic scale the extent of gene flow among *E. puteanus* populations was higher than among *Dolichopoda* populations, whereas the degree of population structuring in *E. fragilis* is undoubtedly

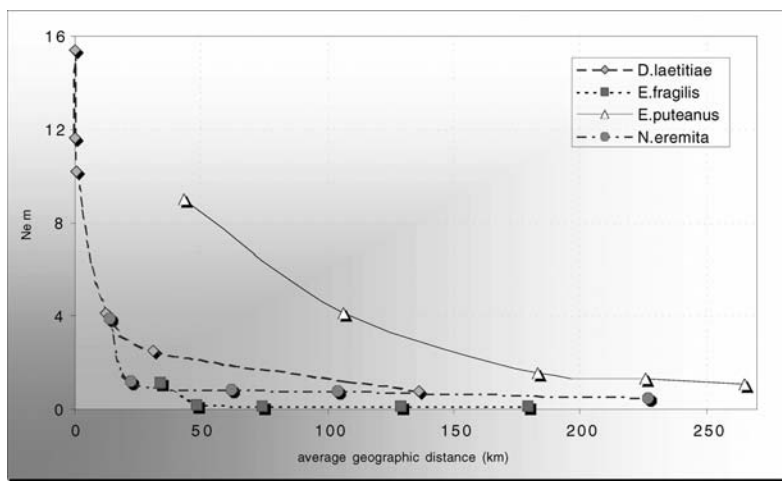


FIGURE 1 The amount of gene flow ($N_e m$), as measured on the basis of the present patterns of genetic differentiation (Wright's F_{ST}), was estimated for multiple combinations of populations at different geographic scales, by considering geographic windows increasingly wider. The four curves outline the trends in *Dolichopoda laetitia*, *Euhadenoecus fragilis*, *Euhadenoecus puteanus* and *Nesticus eremita*.

more than in both *D. laetitia* and *E. puteanus*. Population fragmentation and geographical isolation in *E. fragilis* have already proceeded to a very large extent, because almost irrelevant gene flow is detectable at any scale.

This outcome is in agreement with the well-known features of these three species. Both American species are found in caves, but *E. puteanus* also commonly occurs outside caves in forest habitats, whereas *E. fragilis* is a strictly cavernicolous species inhabiting a patchy limestone area. *Dolichopoda laetitia* shows halfway troglomorphic habits with respect to the two American species. Moreover, troglomorphic features are more advanced in *E. fragilis* than in *E. puteanus*, with *D. laetitia* intermediate both in the degree of leg elongation and in the rate of pigment reduction. The general emerging picture corroborates the idea that gene flow with epigeal populations prevents or retards adaptation to the cave habitat, and that isolation is an important prerequisite for the evolution of troglomorphic adaptations.

In the European spider species, *N. eremita*, the relation between N_m estimates and geographic distance was similar to that observed for *D. laetitia*. *Nesticus eremita* is a widespread troglomorphic species, colonizing not only natural limestone caves, but also artificial caves, cellars, and some epigeal habitats, just like *D. laetitia*. Moreover, this spider can move outside caves and disperse to some extent by means of ballooning at juvenile stages. Therefore both present and past gene flow can explain the pattern of genetic differentiation between populations, which typically reflects a pattern of isolation by distance.

The comparison to the examined cricket species indicates that the dispersal ability for *N. eremita* is lower than that for *E. puteanus*, perhaps lesser than for *D. laetitia*, but undoubtedly higher than for *E. fragilis*. The study cases examined here proved that different cave species, even if closely related, could show different levels of isolation, fragmentation, and dispersal, depending on both extrinsic obstacles to gene flow and intrinsic properties of organisms (Fig. 1).

In some cases, different molecular markers, used to investigate population genetic structures, or technical procedure of data analysis may lead to slightly different conclusions or may make the comparison of patterns of structuring difficult. An example is provided by a species group of Appalachian cave spiders, *N. tennesseensis* "complex," where population genetic structure was investigated using mtDNA sequence data (ND1 gene). Hedin, in a 1997 paper, described high sequence homogeneity within populations with large divergences between populations for all species analyzed. The revealed pattern was consistent with an ancestral interbreeding population, which, at some time in the past, was fragmented into several habitat-limited subpopulations. Subsequent to fragmentation, the exchange of migrants among subpopulations became extremely restricted to nonexistent. The conclusion was that Appalachian *Nesticus* species are characterized by similar and essentially complete population subdivision regardless of difference in species

habitat preference (surface, cave-dwelling, troglomorphic). However, this outcome is in disagreement with many other studies on cave animals, essentially based on allozyme data, where troglomorphic species showed lower rates of gene flow than troglomorphic or epigeal species. Hedin underlined that mtDNA, which is maternally inherited, could reveal a reduced amount of gene flow with respect to estimates based on nuclear loci. This would explain the discrepancy between levels of gene flow estimated in the American species of *N. tennesseensis* complex and the European *N. eremita*. In addition, Hedin suggested that studies on population structure based on allozymes have to take into account that populations currently exchanging no genes can still share ancestral allozyme polymorphisms, i.e., the calculated extent of gene flow could be actually interpreted as the trace of historical gene flow between populations, as already outlined.

SURFACE VS. UNDERGROUND DISPERSAL ROUTES

How can extrinsic and intrinsic factors interact to influence patterns of structuring populations? An additional example is useful. The genetic structures of populations were compared in two pairs of prey-predator species in two regions of Kentucky: (1) the cave cricket, *Hadenoeus subterraneus*, and the carabid beetle *Neaphaenops tellkampfi*, which is a predator of *Hadenoeus* eggs and (2) the cave cricket, *H. cumberlandicus*, and its egg predator *Darlingtonia kentuckensis*. The two regions where the species occur show different extents of fragmentation in the karst cover, with the latter much more dissected into relatively smaller limestone fragments. This difference is expected to affect migration via underground routes more than surface routes, because substantial forest cover was almost continuous in both regions, at least in historical times. This is why population fragmentation and divergence could be strongly affected by extrinsic barriers. Results from allozymic analyses showed a similar overall degree of genetic structuring in the two cave cricket species (average F_{ST} s were 0.58 and 0.46 in *H. subterraneus* and *H. cumberlandicus*, respectively). On the other hand, although *N. tellkampfi* populations showed a degree of genetic structuring ($F_{ST} = 0.56$) similar to cricket species, *D. kentuckensis* populations displayed much higher values of genetic structuring ($F_{ST} = 0.96$, $N_m = 0.01$), suggesting that some of them are already evolving into reproductively isolated species.

POPULATION STRUCTURE IN AQUATIC TROGLOBITES: HYDROLOGICAL ROUTES VS. ADAPTIVE DIVERSITY

In aquatic organisms, hydrologic relationships appear to reflect population connectivity and gene flow much better than geographic distance. In some cases populations from different caves exposed to the same groundwater aquifer may show no trace of genetic differentiation, suggesting a single

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panmictic gene pool, as reported in several crustaceans and fish. In other cases, strong population subdivisions occur even over very short geographic distances.

The crustacean, *Gammarus minus*, has been the object of several detailed investigations by D. Culver, T. Kane, and D. Fong (1995). This freshwater and cave-dwelling amphipod species is found in caves throughout its range, but morphologically highly modified cave populations are found only in two relatively small regions of Virginia and West Virginia in the United States. Populations of *G. minus* reside in resurgences and related subsurface basins; both in cave stream habitats and occasional openings to the surface (karst windows).

Genetic analysis of allozyme polymorphisms pointed out that the hydrological relationships among populations have largely determined the levels of genetic differentiation. Actually, cave and karst-window populations within a given basin are similar to each other. Different resurgence populations show similar genetic characters as well; genetic similarity between populations is by basin, and not by habitat. As a consequence, F-statistics suggest little if any gene flow between basin and resurgence, and little if any gene flow among populations of different basins.

On the other hand, the analysis of morphological variation in *G. minus* generated a different pattern. In this case, populations are similar by habitat rather than by basin. Populations from cave and resurgence are markedly different, and karst-window populations exhibit a wide range of eye sizes, from very small (a troglomorphic character) to the sizes seen in resurgence populations. Both troglomorphic feature variations and genetic structure of populations suggest independent invasion of subsurface basins, and the overall similarity of eyes in cave populations relative to resurgence populations identify a role for natural selection.

A significant correlation between allozyme and morphological distances was also found, suggesting that morphological variation among populations is largely influenced by evolutionary history. However, there is no significant path linking habitat and genetic distances, indicating that selection has little influence on genetic structure. The significant path linking habitat distances and morphological distances does indicate that selection has a strong effect on morphological variation among populations. A series of several interrelated analyses led to the conclusion that *G. minus* is actually a species complex; populations in resurgence habitats constitute one species, and populations in different basins may be as many troglomorphic species as independent isolations occurred in different basins.

POPULATION STRUCTURE IN AQUATIC TROGLOBITES: HISTORICAL DETERMINANTS AND SECONDARY CONTACT

In several instances an apparent population structuring can be determined to some extent by a genetic admixture

between already differentiated gene pools. In tropical karst areas rapid evolution of drainage basins combined with tectonic events may alter connections of surface and cave streams, thus producing either isolation or secondary contact between biota adapted to different conditions. The Cueva de Los Camarones in Chiapas, Mexico, offers an excellent example of such situations. Two undescribed species of *Procambarus* crayfish belonging to the *P. mirandai* species group inhabit the subterranean stream. They were roughly distinguishable only by comparing extreme phenotypes, ranging from dark, thick, eyed, surface-dwelling-like individuals to light, elongate, micro-ophthalmic, cave-dwelling-like individuals. Analyses of allozyme polymorphisms and morphometry were performed to enlighten evolutionary relationships among individual crayfish and to explain patterns of microgeographic variation previously revealed along the cave stream. Results from multivariate morphometric analyses showed a real discontinuity between the two species mainly determined by the shape of the rostrum, chelae, and telson. Moreover, these same characteristics exhibited clinal variation within the less cavernicolous species. The genetic structure of the two species was investigated at 23 enzyme loci, revealing unusually high levels of heterozygosity in both species. Results of analyses on individual allozymic profiles corroborated morphometric results, yielding a genetic distance between the two gene pools of $D = 0.26$. Due to the occurrence of alternative alleles, we could quantify patterns of introgression revealing absence of F1 individuals and asymmetric gene flow between the two species. In the light of these data, the observed microgeographic variation in morphology within one of the two species, as well as the occurrence of aberrant phenotypes, could be interpreted as the outcome of introgression.

POPULATION STRUCTURE WITHIN A SINGLE CAVE

A study was carried out on a *Dolichopoda* population within Grotta delle Piane located in Central Italy, in the Umbria region, at altitudes between 200 and 275 m. It is a karst system developing according to three main tectonic fractures, following different directions — north south, east west, and north/west-south/east. The subterranean karst mainly follows the direction of the faults, originating an entangled labyrinth constituted by overlapping horizontal planes. The cave presents four main entrances and, along with other organisms, it is inhabited by *D. laetitia*, located near the entrances.

To test the hypothesis of a nonrandom distribution of *Dolichopoda* genotypes, the population has been sampled in six near but distinguishable sections where significant aggregates of crickets had been detected. Sectors 1, 2, and 3 are located in the upper level, while sectors 4, 5, and 6 are in the lower one. Population size was evaluated seasonally by mark-recapture. Study samples were classified according to

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their location and age as juveniles, adolescents, and adults. Adults were mostly present during the summer season and overall population size ranged between 1766 (autumn estimates) and 2095 (summer estimates).

Trophic niche was also characterized by means of fecal content analysis. Seven categories of items were determined: arthropod remains, moth scales, bat hairs, rodent hairs, green plant remains, vegetable fibers, and fungi. Correspondence analysis, carried out on the item proportions scored for the six sectors of the cave, showed a differentiation of sector 1 sample with respect to all the others. The subpopulation inhabiting sector 1 was characterized by the dominance of plant matter and fungi in diet. Genetic structure was analyzed at six polymorphic allozyme loci (i.e., *Aph-1*, *Est-1*, *Est-2*, *Gpi*, *Me-3*, *Np*, and *Pgm*). Allele frequencies and heterozygosity estimates were calculated for each of the six considered subpopulations. Multivariate analyses carried out on allele frequencies suggested a clear differentiation of subpopulations inhabiting sectors 4 and 5 between each other and from all the others. *Aph-1* and *Pgm* loci appeared to be chiefly responsible for this ordination. Interestingly, these loci also showed significant deviation from Hardy-Weinberg expectations. The amount of genetic structuring as evaluated by the F_{ST} index was equal to 0.056 if all loci were considered.

In order to estimate levels of gene flow among different subpopulations, F_{ST} pairwise comparisons were considered, excluding from the analysis *Aph-1* and *Pgm* as potential loci under selection. To compare results from mark-recapture experiments and from genetic analysis, the different sectors of the cave were grouped as follows: group A, including sector 4; group B, including sectors 5 and 6; and group C, including sectors 1, 2, and 3. In Fig. 2 such indirect estimates of gene flow between the different sectors of the cave are illustrated. Values were of the same order of magnitude as

direct estimates resulting from mark-recapture experiments performed ad hoc for comparative purposes. Similarly to the following example, it seems that natural selection, by means of nonrandom distribution of different genotypes, is the process implied in shaping population structure in the Piane Cave.

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POPULATION STRUCTURE AND HABITAT HETEROGENEITY

The hypothesis that habitat heterogeneity affects population structure was investigated in the *D. laetitia* population inhabiting the Cerveteri's Etruscan necropolis near Rome, Italy. This is a well-known necropolis extending throughout a roughly elliptical area of 70 hectares. The necropolis includes approximately 300 tombs of different sizes and location. Small colonies of *Dolichopoda* inhabit most of these tombs raising the following questions: How many *Dolichopoda* populations inhabit the necropolis? Is there a case for a metapopulation (i.e., a population of populations) or, alternatively, for a unique population? To what extent does this peculiar habitat structure affect the population genetic structure?

A. Sansotta and the authors of this article faced these questions by means of a long-term series of ecological and genetic investigations to measure the dispersal ability of crickets across tombs, to estimate the effective population size, to determine the amount of heterozygosity of the population, and to assess whether *Dolichopoda* genotypes are randomly distributed throughout the necropolis.

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Extensive fieldwork was carried out by means of mark-recapture techniques based on individual tagging. Both allozyme polymorphisms and RAPD-DNA markers were employed to carry out genetic analysis.

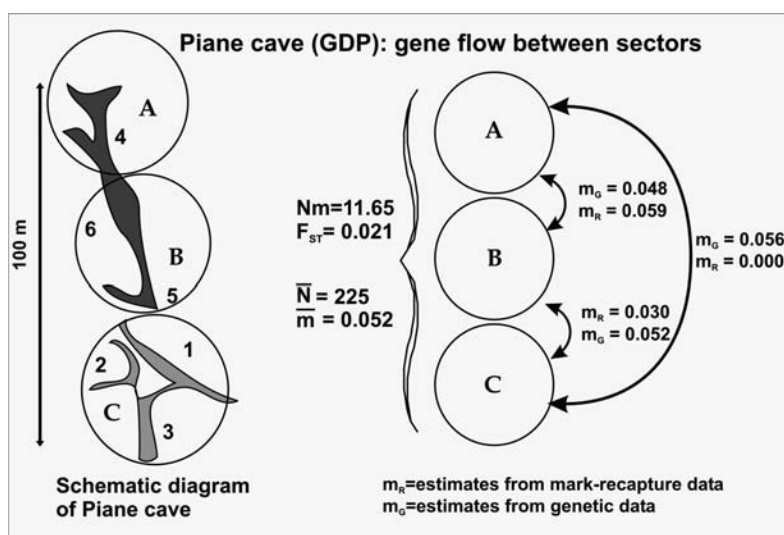


FIGURE 2 Estimates of gene flow (Nm) between different sectors of the Piane cave calculated from F_{ST} values (F-statistics, Wright 1965). N = population size; m = migration rate.

As a first result from this study, crickets revealed remarkable dispersal ability as they proved to move significantly across tombs to such an extent that the necropolis can be considered as the home range for a panmictic population as a whole. Second, a population sample collected across the necropolis exhibited relatively high values of heterozygosity (Ho = 0.218; He = 0.244) compared to other *Dolichopoda*. Third, individuals carrying definite allele combinations (genotypes) were located preferentially in some tombs within the necropolis to such an extent that genotypes in different partitions of the necropolis were not randomly distributed, but were significantly associated to some cave ecological descriptors, namely temperature.

In summary, these crickets appeared to respond to the necropolis spatial and environmental heterogeneity by means of habitat choice expressed by different genotypes. Therefore, multiple niche selection associated to habitat choice appears to be the most probable selective process to explain the observed high level of genetic polymorphism.

POPULATION STRUCTURE AND FOOD RESOURCE HETEROGENEITY

The Frasassi Cave System is located in Central Italy, on the Adriatic side of the Apennine Mountains. This karst system is developed in at least four main horizontal levels. Each level shows a complex pattern, strongly influenced by faults and by a hydrogeological setting. The system is characterized by the occurrence of sulfide streams in some sections and by deposits of guano in others. The oniscidean isopod, *Androniscus dentiger*, is the most abundant species in different habitats in the Frasassi caves. G. Gentile and S. Sarbu (2000) studied the possible occurrence of genetic structuring in this species in population samples collected at different sites by means of F_{ST} analyses based on 18 allozyme loci. Among other samples collected throughout different caves in this system, three neighbor samples were collected within Grotta del Fiume (i.e., Guano room, Green Lake room and along a lateral sulfide section).

Results indicated departures from Hardy-Weinberg equilibrium at several loci, generally due to heterozygote deficiencies. The F_{ST} value was 0.180, indicating a level of genetic structuring comparable to values found among geographically distant populations of the same species. However, even by removing the Grotta del Mezzogiorno sample from the analysis, the amount of population subdivision remained unexpectedly high ($F_{ST} = 0.180$), especially considering the neighborhood of sampling locations within the same cave. Such an extent of population structure implies very low levels of gene flow, as shown between the Green Lake room and the lateral sulfide section, although each of these demes shows a limited gene exchange with the subpopulation from the Guano room. Interestingly enough, compared to other samples, individuals collected in the sulfide section were proved to feed on different types of resources such as chemo-

autotrophically synthesized food, as established by stable isotope ratio analysis. Again we are facing a situation where natural selection could be involved in promoting and maintaining population structuring in *A. dentiger* in the Grotta del Fiume.

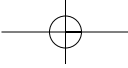
Caves are often expected to be very homogeneous environments as a result of their physical parameters and habitat stability. This may not necessarily be true, at least in instances where localized trophic resources may convert a cave into a patchy habitat, constraining colonies to set around resources, in spite of the potential dispersal capability of individuals. If trophic resource heterogeneity remains stable over time, we could expect that genetic polymorphism is maintained by natural selection. This interpretation explains why several cave-dwelling organisms show relatively high heterozygosity levels notwithstanding their reduced population sizes.

GENETIC VARIABILITY, POPULATION SIZE, AND NATURAL SELECTION

The amount of genetic variability expressed by troglobitic populations is a controversial issue. In fish, for example, a recent study based on RAPD markers suggests that hypogean populations of balitorid fish have lower genetic variability than related surface populations. Similar results were obtained from allozymic studies in the Mexican cavefish *Astyanax fasciatus*, amblyopsid fishes from North America, and a trichomycterid species from Venezuela. The decreased genetic variation observed was reputedly consistent with the expectation that the troglobitic fish have smaller population sizes than the epigeal species. Limited to single caves and cave systems, they supposedly have small population sizes. However, a study of hypogean cyprinid fish from Somalia showed that populations of *Phreatichthys andruzzii*, a troglomorphic stygobiont species, are more heterozygous than most epigeal cyprinid species studied thus far. In addition, when comparing this species with its closest relative, *Barbopsis devechii*, a micro-ophthalmic, less specialized fish also occurring in the groundwaters of Somalia, statistically significant higher heterozygosity values were revealed (He ranging from 0.046 to 0.062 in *Phreatichthys* and from 0.014 to 0.020 in *Barbopsis*). Due to the lack of appropriate population estimates, we cannot test the hypothesis that these differences are related to population sizes. In *Phreatichthys* it has been hypothesized that selective advantage for individuals with high heterozygosity could be involved. Negative correlations have been found in various organisms between individual heterozygosity levels and fitness components such as rate of oxygen consumption, energy requirements for maintenance, etc. These and other metabolic features may represent adaptations to low-energy-input conditions in troglobites and susceptibility to affect selective advantage to a considerable extent. Comparing population structures of *Phreatichthys* to *Barbopsis*, it was revealed that the former is strongly structured into

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Orthoptera	No. species	No. pops	Average He	Range of He
<i>Noctivox</i>	1	1	0.180	—
<i>Longuripes</i>	1	1	0.253	—
<i>Hadenoecus</i>	5	18	0.078	0.020–0.130
<i>Euhadenoecus</i>	4	24	0.064	0.030–0.110
<i>Ceuthophilus</i>	1	7	0.026	—
<i>Dolichopoda</i>	10	52	0.144	0.056–0.209
<i>Troglophilus</i>	9	19	0.060	0.000–0.178

genetically isolated populations while consistent gene flow maintains genetically homogeneous in even geographically distant populations of *Barbopsis*, indicating an earlier cave isolation of *Phreatichthys* populations. On the contrary, *Barbopsis* could have experienced a longer direct contact with their epigeic relatives. Because isolation in the cave habitat by means of breakdown of gene flow with epigeic populations is a prerequisite to enhance adaptation of cave populations, it could be speculated that old established cave populations have a higher probability of expressing fitness-related features in their gene pools such as increased heterozygosity.

The tenet that troglobites or troglomorphic cave dwellers have low genetic variability may not be generally applicable. A comparison between troglomorphic and nontroglomorphic cave crickets like *Dolichopoda* vs. *Troglophilus*, *Hadenoecus* vs. *Ceuthophilus*, and *Longuripes* vs. *Noctivox* leads to the invariable result that the former have higher heterozygosity than the latter (Table I).

Moreover it can be seen that, among other taxa, several stygobiontic crustaceans such as amphipods and isopods as well as troglobitic beetles show evidence for very high levels of heterozygosity (Table II).



Niphargus longicaudatus. Photo by V. Sbordoni

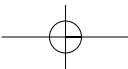


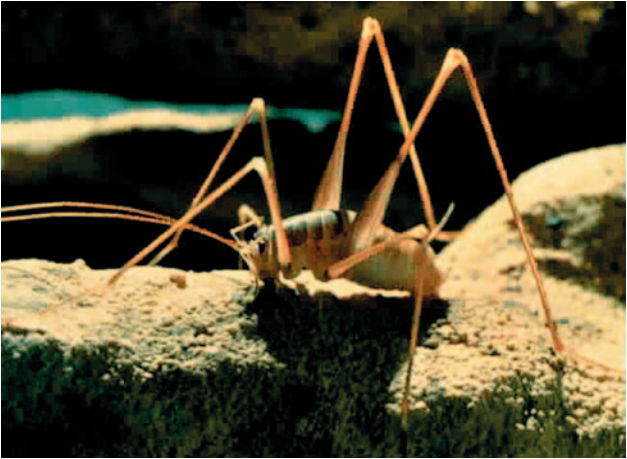
Leptodirus hohenuwarthi. Photo by V. Sbordoni

TABLE I TTL Table Title

Crustacea	No. species	No. pops	Average He	Range of He
Amphipoda				
<i>Crangonyx</i>	1	6	0.118	—
<i>Gammarus</i>	1+	8	0.108	0.075–0.130
<i>Niphargus</i>	4	9	0.274	0.104–0.347
Isopoda				
<i>Androniscus</i>	5+	34	0.102	0.027–0.178
<i>Trichoniscus</i>	2+	12	0.089	0.034–0.218
<i>Stenasellus</i>	4	13	0.079	0.000–0.196
<i>Proasellus</i>	1+	3	0.084	0.037–0.148
<i>Typhlocyrolana</i>	2	3	0.040	0.029–0.061
<i>Monolistra</i>	3	3	0.280	0.261–0.316
Coleoptera				
Carabidae				
<i>Duvalius</i>	2	3	0.114	0.100–0.141
<i>Neaphaenops</i>	1	8	0.192	0.173–0.222
<i>Darlingtonia</i>	1	10	0.009	0.000–0.040
<i>Pseudanophthalmus</i>	3	13	0.094	0.053–0.170
Cholevidae				
<i>Bathysciola</i>	1	1	0.121	—
<i>Speonomus</i>	5	44	0.113	0.060–0.192
<i>Orostygia</i>	2	2	0.165	0.133–0.198
<i>Leptodirus</i>	1	1	0.168	—
<i>Promaphagus</i>	1	6	0.056	0.012–0.099

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One *Dolichopoda* specimen. Photo by V. Sbordoni



Androniscus dentiger. Photo by G. Gentile

These findings can hardly be explained by large population sizes, but they could reflect the existence of genetic polymorphisms maintained by balancing selection. Interestingly, high heterozygosity levels have been revealed mainly in small arthropods, which appear to perceive the environmental patchiness as coarse grained and therefore favorable in which to adapt to habitat and/or resource heterogeneity by means of a multiple niche polymorphism strategy.

Further Reading

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Somali cave fish:

Barbopsis devecehii

Phreatichthys andruzzii

Photos from Ercolini, Berti, Chelazzi & Messana. 1982. "Researches on the phreatobic fishes of Somalia: achievements and prospects" *Monitore Zoologico Italiano*, N.S. Suppl., 17, 9: 219–241.

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