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Are troglobitic taxa troglobiomorphic? 
A test using phylogenetic inference

Laure Desutter-Grandcolas *

SUMMARY

Obligate cave dwelling organisms are frequently characterised by a peculiar morphological syndrome, named troglomorphosis or troglobi​omorphosis. This hypothesis, which deals with the evolutionary influence of the subterranean environment on cave organisms is far from being universally accepted. Yet it has been adopted by many authors and is often included in the definitions of the current classification of cave taxa.

In this paper I present a test of the troglobiomorphosis hypothesis, using the case study of the cricket clade Amphiacustae (Orthoptera, Grylloidea, Phalangopsidae). Such a test preliminarily requires that observations of the habitat of the taxa (achieved on present-day populations) are clearly separated from hypotheses on the evolutionary transformations of cave taxa (troglobiomorphosis hypothesis s. str.). The evolutionary hypotheses on troglobite morphology are tested using phylogenetic inference, that is by parsimoniously mapping the states of several morphological characters (eye size, body colour, relative hindleg size) onto the cladogram of the Amphiacustae.

According to these phylogenetic analyses, the troglobiomorphosis hypothesis is corroborated by the patterns reconstructed for eye size and body coloration characters, but is refuted by the patterns built for hindleg size.

INTRODUCTION

In the numerous studies dealing with the evolution of troglobitic life, troglobitic taxa are frequently defined as having a peculiar morphological syndrome named “troglobiomorphosis” (Barr, 1968; Ginet & Decu, 1977; Howarth, 1983; Barr & Holsinger, 1985; Camacho, 1992) or “troglobio-

1 As data on the physiology and life history strategies of cave dwelling organisms are accumulating, although on very few species yet, characteristics other than morphological are now included in the «troglobiomorphic» syndrome (Christiansen, 1992; Thibaud, 1994). Such data are not considered here, but all the conclusions drawn from my morphological analysis apply to them, especially those concerning methodological requirements.

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morphosis" (Juberthie & Decu, 1994). A pale coloration, an increase of appendage size (legs, palpi, antennae...), the reduction or the loss of eyes and wings are often given as the most conspicuous morphological changes, among others, shown by “true troglobites”. As emphasised by Bellès (1991: 325) for example, “The most peculiar cave-adapted group is, of course, that of troglobites, which have been typified by a series of morphological features, including loss or reduction of eyes, wings and cuticular pigments, and an increase of sensorial devices, often accompanied by the development of longer appendages and a more slender body form: the so-called “troglobiomorphic” characters” (italics mine). According to most authors, this is a facet of the “regressive evolution” of cave dwelling organisms (Vandel, 1964; Barr, 1968; but see Howarth, 1987; Botosaneanu & Holsinger, 1991).

There is no existing consensus concerning the degree of generality of the troglobiomorphosis hypothesis and several authors have criticised the tendency to consider as troglobites only the taxa presenting these morphological peculiarities. As Vandel (1964: 29) stated: “Aucun critère morphologique ne peut être tenu comme strictement caractéristique des cavernicoles [restricted here to troglobites]. Tout ce que nous pouvons affirmer, c’est que certaines manifestations (en particulier, la dépigmentation et l’anophthalmie) sont statistiquement plus fréquentes chez les cavernicoles que chez les épiéges” (square brackets mine). Similarly Culver (1982: 36) remarked that: “Many troglobophiles have no known surface populations and are classified as troglobophiles only because they show little sign of regressive evolution”. Despite such facts and comments, the troglobiomorphosis hypothesis has become more and more generally used. It has been included in the most commonly used classification of cavernicolous taxa (Racovitza, 1907; Ginet & Decu, 1977; Bellès, 1991; Peck & Finston, 1993, ...), although this classification was initially based solely on ecological grounds (Schiner, 1854; Vandel, 1964), or has even been used as the main criterion for classification (Christiansen, 1962).

As noted by several authors, however, this current opinion is a confusing mixture of ecological and evolutionary hypotheses, especially of those hypotheses dealing with the habitat of the taxa, their phenotypic responses to a subterranean habitat and their evolutionary transformations (Barr & Holsinger, 1985; Bellès, 1991; Matile, 1994; Desutter-Grandcolas, 1997c). As a result none of these hypotheses can be thoroughly tested, because each hypothesis is directly linked to several others and none is independent (Eldredge & Cracraft, 1980; Grandcolas et al., 1994). How can we test if troglobiomorphy characterises obligate cave dwelling taxa if we a priori assume that troglobitic taxa are troglobiomorphic? Similarly how can we assume that a character has been modified because of cave colonisation
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if the ancestral state of this character in the clade to which the troglobitic taxa under study belong is unknown?

In order to test if troglobitic taxa are troglobiomorphic, it is necessary beforehand to clearly separate the hypotheses concerning the habitat of the taxa, and those dealing with their evolutionary transformations. The only alternative would be a non-scientific faith in current opinion and an abandon of any scientific approach on cave life analysis. Habitat characterisation results from present-day observations and is achieved through population studies. On the contrary, studies of the evolutionary history of the taxa are performed per natura in a historical perspective: this implies that the characters under study are analysed in a phylogenetic perspective (Carpenter, 1989; Brooks & McLennan, 1991; Grandcolas et al., 1994). Habitat characterisation on one hand and evolutionary transformations of the taxa on the other are two different scientific questions, which can be studied by two different kinds of approaches, population biology and comparative biology respectively (Peck, 1981; Grandcolas et al., 1997).

Let us consider first the problem of habitat characterisation. An immediate consequence of the above is that the current classification of cavernicolous taxa needs to be modified to allow the description of the habitats of the taxa without presuming their evolutionary transformations. I have analysed this problem in another paper and proposed to classify the taxa exclusively according to their behavioural ecology (Desutter-Grandcolas, 1997c) only those taxa which live and reproduce in the subterranean environment without leaving it, should thus be qualified as troglobitic. Other taxa would be classified according to their own main habitat (straminicolous, cavicolous, dendrophilous, etc., see for example Lincoln et al., 1982), even though they may appear more or less accidentally or regularly in caves, which may represent a substitute of their habitat. For example, many nocturnal cavernicolous crickets (Orthoptera, Orylloidea) use caves to hide during the day; they have been erroneously qualified as cavernicolous or troglophiles (Vandel, 1964; Leroy, 1967), but their natural habitat includes burrows or hollow trees, and they have no peculiar link with subterranean habitats. The proposed classification no more recognises so-called trogophilic or troglaxenous taxa, a source of ambiguity in many previous classifications (see comments in Christianson, 1962; Barr, 1968; Howarth, 1983; Barr & Holsinger, 1985; Peck, 1990; Culver, 1982; Thibaud, 1994). Also it completely separates the habitat from the evolutionary history of the taxa. In fact it only requires that observations are made in the natural environment, outside caves, to check for the habitat of the taxa. This classification has been used for example to clarify the list of cave living crickets (Orthoptera, Grylloidea) (Desutter-Grandcolas, 1997a; see also Desutter-Grandcolas, 1993, 1995a) and is adopted in the present paper.
The second problem is to study the evolutionary transformations of troglobitic taxa. In order to build sound hypothesis on character transformation, one needs to know which are the ancestral states of the characters and how the characters have evolved in the clade under study (polarity of change). Given these evolutionary patterns, one should be able to answer the following questions: have troglobitic taxa kept the ancestral states of the characters or not? And have character modifications co-occurred with cave colonisation? Such an analysis can be achieved only by using the method of phylogenetic inference, which consists in using a phylogeny, built according to cladistics, to reconstruct the ancestral states and the evolutionary transformations of taxa features (Andersen, 1979, 1994; Wanntorp et al., 1990; McLennan, 1991; Packer, 1991; Grandcolas et al., 1994; Andersen & Weir, 1994).

Phylogenetic inference is further employed to test hypotheses in evolutionary biology (Eldredge & Cracraft, 1980; Coddington, 1988, 1990; Carpenter, 1989; Brooks & McLennan, 1991; Ross & Carpenter, 1991; Grandcolas et al., 1994, 1997), by comparing the transformation pattern an evolutionary hypothesis predicted with the phylogenetic pattern derived from cladistic analyses: if both patterns fit, the hypothesis is corroborated in the case under study; on the reverse, if both patterns do not fit, the hypothesis is invalidated. The comparison, by analogy, of as many case studies as possible may allow to generalise the tested hypothesis, to modify it or to reject it.

There is a growing number of studies in comparative biology that use phylogenetic inference to reanalyse a large array of evolutionary problems (Andersen, 1979, 1994; Coddington, 1988, 1990; Carpenter, 1989; Wanntorp et al., 1990; Packer, 1991; Ross & Carpenter, 1991; Siddall et al., 1993; Desutter-Grandcolas, 1993, 1994b, 1996b, 1997b; Grandcolas, 1993, 1996, 1997b; Grandcolas & Deleporte, 1996; Andersen & Weir, 1994; Siddall & Burreson, 1995; ...). Troglobitic evolution is beginning to be reanalysed that way. Current hypotheses of the factors responsible for cave life evolution (Desutter-Grandcolas & Grandcolas, 1996) and, more generally, the main concepts of current theory of troglobitic evolution (Desutter-Grandcolas, 1997c, have been reevaluated using phylogenetic inference.

I will present here a phylogenetic analysis of the troglobiomorphosis hypothesis in the cricket clade Amphiacustae. This clade has previously been studied for habitat evolution (Desutter-Grandcolas, 1993, 1994b) and for the evolution of wings and stridulatory apparatus in males (Desutter-Grandcolas, 1995a, 1997d): it has then been shown that cave colonisation and wing modification did not co-occur and that the loss of acoustic communication did not appear subsequently to cave colonisation. These results partly invalidated the hypothesis of troglobiomorphosis. I will here resume
this troglobiomorphic analysis and test for the evolution of body coloration, eye size and hindleg size in the Amphiacustae.

MATERIAL AND METHOD

Material

The Amphiacustae are distributed in Central America and in the West Indies (Desutter-Grandcolas, 1993). The monophyly and internal phylogeny of this clade have been assessed by the cladistic analyses of morphoanatomical characters (Desutter-Grandcolas, op. cit. and Fig. 1A). The phylogeny was worked out at the genus level, each genus being defined as a monophyletic entity. The present analyses were then achieved at this same level, and 67 species (253 specimens) over the 69 described up to now in this clade have been taken into consideration to precisely define the traits (cf. infra) of each genus (Desutter-Grandcolas, 1993, 1994a, 1995b, 1997e; Desutter-Grandcolas & Otte, 1997).

The habitats of the taxa have been assessed in a previous paper using data found in the literature and personal observations in the field (see Desutter-Grandcolas, 1993). Two different habitats were then defined: 1/ straminicolous - cavicolaous (epigean) and 2/ troglobitic. Only one parsimonious scenario was derived to account for habitat distribution among the Amphiacustae: it implied two independent evolutions toward a subterranean life and one reversal toward an epigean way of life (Desutter-Grandcolas, 1993, 1994b, 1997c and Fig. 1B).

Fig. 1 – Phylogeny (A) of Amphiacustae and reconstructed scenario (B) of their habitat evolution (modified from Desutter-Grandcolas, 1993, 1994b). Ancestral state in a grey frame; attribute change indicated by a large bar and a white frame.
All the genera were found homogeneous for their habitat, except *Noctivox*. In the present paper, the following taxa were then considered in the character analyses: 1/ epigean taxa: *Amphiacusta, Cantrallia, Leptopedetes, Nemoricantor*, epigean *Nocrivox* (*Noct. epig.*) and *Prolonguripes*; 2/ troglobitic taxa: *Arachnopsita, Longuripes, Mayagryllus* and troglobitic *Noctivox* (*Noct. troglo*, including two closely related taxa). The internal phylogeny of *Noctivox* being still unknown, the subdivision of *Noctivox* used here in character analyses was not taken into account as such in the Amphiacustae cladogram.

*Method for troglobiomorphosis analysis*

In order to test the hypotheses of troglobiomorphosis, several traits have been considered, namely the body coloration, the size of the eye and that of the hindlegs. None had been incorporated in the data matrix used to build the phylogeny of Amphiacustae for lack of reasonable argument to assess primary homology (De Finna, 1991). For each trait, several states have been defined in the taxa under study and mapped onto the Amphiacustae phylogeny (optimisation procedure), using Wagner parsimony extended to multistate traits (Farris, 1970; Fitch, 1971). Hypotheses of the ancestral states of the traits and of their subsequent transformations were thus derived and compared with the patterns implied by troglobiomorphosis hypotheses (Carpenter, 1989; Brooks & McLennan, 1991; Grandcolas et al., 1994).

*Attributes and attribute states definitions*

States of the features analysed here are the following (Table 3):

1/ **Body coloration**: d, dark; p, pale. All the groups considered in the analysis are homogeneous for this character.

2/ **Eye size**: l, large; s, small. All the groups considered in the analysis are homogeneous for this character (Fig. 2-11).

3/ **Relative size of hindlegs**. Two ratios have been used to account for hindleg size: rat1: Length of hindfemur / Length of the pronotum, and rat2: Length of hindtibia / Length of the pronotum.

The length of the pronotum is considered a good indicator of the size of a specimen, rather than its width, which appears influenced by the presence and development of the wings, and rather than the whole body length, which is sensitive to the actual condition of the specimens (dry or in alcohol) and to their physiological state when collected (especially for females).
Figs. 2-11 – Heads of amphiarct taxa (right lateral view) showing the eye size. 2, Amphicusta annulipes; 3, Noctivox sanchezi; 4, N. bolivari; 5, Cantrallia huasteca; 6, Leptopedetes chiriquensis; 7, Nemoricantor aztecs; 8, Arachnopsita usumacinta; 9, Longuripes sordoni; 10, Prolonguripes giganteus; 11, Mayagryllus apterus. Scale 1 mm. Troglobitic taxa identified with a black arrow.

Ratio values were computed directly using the measurements of each specimen and not only the minimum, maximum and mean values indicated in the descriptions of the species (DesutterGrandcolas, 1993, 1994a, 1995b, 1997e; Desutter-Grandcolas & Otte, 1997).

Both rat1 and rat2 are continuous variables. To define the states of each attribute, I performed two-sample Wilcoxon tests between pairs of taxa, with a confidence interval of 95%. These analyses clustered together the taxa for which ratio value distributions have close median values. Tables 1
and 2 show the results of these tests for rat1 and rat2. According to these results, the following states were defined for these features:

**rat1:** 4 states are defined, clustering the following taxa:

- **state a:** *Cantrallia.*
- **state b:** *Amphiacusta, Arachnopsita, Noct. troglo, Mayagryllus.*
- **state c:** *Nemoricantor, Noct. epig.*
- **state d:** *Leptopedetes, Longuripes, Prolonguripes.*

Figure 12 shows the relative values of these states. The ratios are higher from a to d, indicating a bigger hindfemur size.

**rat2:** 4 states are defined, clustering the following taxa:

- **state a:** *Cantrallia.*
- **state b:** *Amphiacusta.*
- **state c:** *Arachnopsita, Noct. troglo* and *Noct. epig.*
  *Mayagryllus* and *Nemoricantor* present slightly different ratio value distributions and ambiguously cluster with the preceding taxa; they are indicated as (c) in Table 3.
- **state d:** *Leptopedetes Longuripes, Prolonguripes.*

Figure 13 shows the relative values of these states. The ratios are higher from a to d, indicating a bigger hindtibia size.

**Fig. 12 –** Attribute LFIII / Lpron: minimum, maximum and mean values for each taxon, and states used in the phylogenetic analyses. Symbols: ▲: state a; ●: state b; ○: state c; ■ state d. Explanations see text.
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RESULTS

1/ Body coloration

Figure 14 shows the distribution of body coloration states in the Amphiacustae. Only one most parsimonious scenario (3 steps) accounts for the distribution of this two-state feature: it implies a dark ancestral coloration, two independent modifications toward a pale coloration, in Noct. troglo and in the subgroup [(Arachnopsita (Longuripes - Prolonguripes) Mayagryllus], and one reversal toward a dark body colour in Prolonguripes. It appears clearly that all the troglobitic taxa have a pale coloration, while epigean taxa have a dark one, and that colour modification events co-occur with habitat changes. This corroborates the current hypothesis of body colour change (paler coloration) in subterranean environment.

2/ Size of the eyes

Figure 15 shows the distribution of eye size states in Amphiacustae. As for body coloration only one most parsimonious scenario (3 steps) accounts
for this distribution: it implies two independent reductions of eye size, in *Noct. troglo* and in the subgroup [(*Arachnopsita (Longuripes - Prolonguripes) Mayagryllus*], and one reversal toward a larger eye size in *Prolonguripes*. As for body colour, these modifications co-occur with habitat change and corroborate the current hypothesis of eye size reduction in subterranean environment.

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**Fig. 14** - Phylogenetic scenario for body coloration. States: d: dark; p: pale. Names of the taxa and symbols as in Fig.1.

**Fig. 15** - Phylogenetic scenario for eye size. States: s: small; l: large. Names of the taxa and symbols as in Fig.1.
Three most parsimonious scenarios (5 steps) account for the distribution of rat1 states in Amphiacustae (Fig. 16). They all imply that b is the ancestral state and that state a occurred in Cantrallia; they moreover show convergent changes either toward state c or toward state d:

Scenario 1 (Fig. 16A): state c appeared independently in Noct. epig and in Nemoricantor, and state d appeared independently in Leptopedetes and in the subgroup (Longuripes - Prolonguripes).

Scenario 2 (Fig. 16B): state d appeared independently in the subgroups (Longuripes - Prolonguripes) and (Leptopedetes - Nemoricantor), and one subsequent change to state c occurred in Nemoricantor, independently from the appearance of state c in Noct. epig.

Scenario 3 (Fig. 16C): state c appeared independently in the subgroup (Leptopedetes - Nemoricantor) and in Noct. epig, and one subsequent change to state d occurred in Leptopedetes, independently from the appearance of state d in the subgroup (Longuripes - Prolonguripes).

Fig. 16 - Phylogenetic scenarios for hindleg size: LFIII / Lpron. States: see text and Fig. 12. Names of the taxa and symbols as in Fig. 1.
These scenarios show that the evolution of rat1 in Amphiacustae has been complex and that it did not follow cave colonisation. State d thus exists on one hand in the epigean genus *Leptopedetes*, and on the other hand in the troglobitic genus *Longuripes* and its epigean sister genus *Prolonguripes*. On the reverse, the troglobitic species of *Noctivox* have kept the ancestral state of rat1, while the epigean species of this same taxon have evolved toward a higher ratio. The troglobitic taxa *Mayagryllus* and *Arachnopsita* have also kept the ancestral state of rat1.

4/Size of hindleg: rat2 (LTIII / Lpron)

Only one most parsimonious scenario (4 steps) accounts for the distribution of rat2 states in Amphiacustae (Fig. 17). It implies that the ancestral state is state c, and that the following changes have occurred: state b appeared in *Amphiacusta*, state a in *Cantrallia*, and state d independently in *Leptopedetes* and in the subgroup (*Longuripes* - *Prolonguripes*).

If states C and (c) were considered different, the situation would be more complex, with several possible scenarios (6 steps each), the ancestral state being either b, c or d. The conclusions concerning the evolutionary tendencies of rat2 would anyhow be similar.

As for rat1, the hypothesis of troglobiomorphosis is not supported by the reconstructed scenario for rat2. The highest ratio (state d) thus appeared independently in the epigean *Leptopedetes*, and in the troglobitic genus *Longuripes* and its epigean sister genus *Prolonguripes*. On the reverse, other troglobitic Amphiacustae kept the ancestral state of rat2 (*Noctivox* p.p., *Arachnopsita*), or present a slight modification of this state (*Mayagryllus*).

![Fig. 17 – Phylogenetic scenario for hindleg size: LTIII / Lpron. States: see text and Fig. 13. Names of the taxa and symbols as in Fig. 1.](image-url)
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Table 1 – Two-sample Wilcoxon tests for the attribute LFIIII / Lpron. Explanations: see text.

<table>
<thead>
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<th>LFIIII/Lpron</th>
<th>Longuripes</th>
<th>Prolonguripes</th>
<th>Cantrallia</th>
<th>Arachnopsita</th>
<th>Nemoricantar</th>
<th>Mayagryllus</th>
<th>Leptopedetes</th>
<th>Noct. epig.</th>
<th>Noct. troglo</th>
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<td>Mayagryllus</td>
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<td>p=0.0002</td>
<td>W=530.0</td>
<td>W=629.0</td>
<td></td>
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<tr>
<td></td>
<td>p=0.0002</td>
<td>p=0.0388</td>
<td>p=0.0533</td>
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<td></td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
<td></td>
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<tr>
<td>Leptopedetes</td>
<td>W=160.0</td>
<td>p=0.0015</td>
<td>W=127.0</td>
<td></td>
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<td></td>
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<td>p=0.0006</td>
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<td>Noct. epig.</td>
<td>W=678.5</td>
<td>p=0.0016</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>p=0.0000</td>
<td>NS at 0.05</td>
<td></td>
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</table>

Table 2 – Two-sample Wilcoxon tests for the attribute LTIIII / Lpron. Explanations: see text.

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<th>LTIIII/Lpron</th>
<th>Longuripes</th>
<th>Prolonguripes</th>
<th>Cantrallia</th>
<th>Arachnopsita</th>
<th>Nemoricantar</th>
<th>Mayagryllus</th>
<th>Leptopedetes</th>
<th>Noct. epig.</th>
<th>Noct. troglo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphicacusta</td>
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<td>p=0.0000</td>
<td>W=2162.5</td>
<td>W=1769.0</td>
<td>W=1431.0</td>
<td>W=1512.0</td>
<td>W=1347.0</td>
<td>W=1690.0</td>
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<tr>
<td></td>
<td>p=0.0000</td>
<td>p=0.0000</td>
<td>p=0.0056</td>
<td>p=0.0013</td>
<td>p=0.0000</td>
<td>p=0.0023</td>
<td>p=0.0008</td>
<td>p=0.0038</td>
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<td>NS at 0.05</td>
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<tr>
<td>Longuripes</td>
<td>W=1739.0</td>
<td>p=0.9987</td>
<td>W=2711.0</td>
<td>W=1875.0</td>
<td>W=2528.0</td>
<td>W=1568.0</td>
<td>W=2607.5</td>
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<td></td>
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<td>p=0.0000</td>
<td>p=0.0001</td>
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<td>p=0.0933</td>
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<td>NS at 0.05</td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
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<tr>
<td>Prolonguripes</td>
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<td>p=0.0000</td>
<td>W=493.0</td>
<td>W=247.0</td>
<td>W=437.0</td>
<td>W=160.0</td>
<td>W=446.0</td>
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<td></td>
<td>p=0.0000</td>
<td>p=0.0004</td>
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<td>p=0.0710</td>
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<td>NS at 0.05</td>
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<td>NS at 0.05</td>
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<tr>
<td>Cantrallia</td>
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<td>p=0.0000</td>
<td>W=171.0</td>
<td>W=171.0</td>
<td>W=171.0</td>
<td>W=171.0</td>
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<tr>
<td></td>
<td>p=0.0000</td>
<td>p=0.0000</td>
<td>p=0.0000</td>
<td>p=0.0009</td>
<td>p=0.0000</td>
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<tr>
<td></td>
<td>p=0.0000</td>
<td>NS at 0.05</td>
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<td></td>
</tr>
<tr>
<td>Arachnopsita</td>
<td>W=490.5</td>
<td>p=0.0313</td>
<td>W=546.0</td>
<td>W=410.0</td>
<td>W=714.5</td>
<td>W=614.0</td>
<td></td>
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<tr>
<td></td>
<td>p=0.0010</td>
<td>p=0.0002</td>
<td>p=0.0001</td>
<td>p=0.4651</td>
<td>p=0.7053</td>
<td></td>
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<tr>
<td></td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
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<tr>
<td>Nemoricantar</td>
<td>W=189.0</td>
<td>p=0.6307</td>
<td>W=75.0</td>
<td>W=254.5</td>
<td>W=190.0</td>
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<tr>
<td></td>
<td>p=0.0414</td>
<td>p=0.0829</td>
<td>p=0.0786</td>
<td></td>
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<td></td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
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</tr>
<tr>
<td>Mayagryllus</td>
<td>W=345.0</td>
<td>p=0.0194</td>
<td>W=800.5</td>
<td>W=644.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>p=0.0016</td>
<td>p=0.0015</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Leptopedetes</td>
<td>W=134.0</td>
<td>p=0.0018</td>
<td>W=92.0</td>
<td></td>
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<tr>
<td></td>
<td>p=0.0026</td>
<td>NS at 0.05</td>
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</tr>
<tr>
<td>Noct. epig.</td>
<td>W=523.0</td>
<td>p=0.0080</td>
<td></td>
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<td></td>
<td>NS at 0.05</td>
<td>NS at 0.05</td>
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</tbody>
</table>
Table 3 – Distributions of the states of the features studied in this paper. Explanations: see text.

<table>
<thead>
<tr>
<th></th>
<th>Body colour</th>
<th>eye size</th>
<th>LFIII / Lpron</th>
<th>LTIII / Lpron</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphiacusta</em></td>
<td>dark</td>
<td>large</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td><em>Longuripipes</em></td>
<td>pale</td>
<td>small</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td><em>Prolonguripies</em></td>
<td>dark</td>
<td>large</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td><em>Cantrallia</em></td>
<td>dark</td>
<td>large</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td><em>Arachnopsis</em></td>
<td>pale</td>
<td>small</td>
<td>b</td>
<td>(c)</td>
</tr>
<tr>
<td><em>Nemoricantor</em></td>
<td>dark</td>
<td>large</td>
<td>c</td>
<td>(c)</td>
</tr>
<tr>
<td><em>Mayagryllus</em></td>
<td>pale</td>
<td>small</td>
<td>b</td>
<td>(c)</td>
</tr>
<tr>
<td><em>Leptopedetes</em></td>
<td>dark</td>
<td>large</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td><em>Noct. epig.</em></td>
<td>dark</td>
<td>large</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td><em>Noct. troglo.</em></td>
<td>pale</td>
<td>small</td>
<td>b</td>
<td>c</td>
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</tbody>
</table>

**DISCUSSION**

The phylogenetic analyses presented in this paper partly corroborate and partly refute the hypothesis of troglobiomorphosis. In both cases however the patterns of change of attribute states reveal that homoplasies (convergence, reversal) frequently occur in the case under study: these could not have been documented in a non phylogenetic context, for lack of independent argument to assess attribute change polarity (Carpenter, 1989; McLennan, 1991; Grandcolas et al., 1994; Desutter-Grandcolas, 1997c).

In the present analyses, some attribute states are present only in troglobitic Amphiacustae and their evolutionary transformations co-occurred with habitat changes in this clade: they appeared in the taxa which have shifted toward a subterranean habitat (troglobitic *Noctivox* species and the subclade [Arachnopsis - Mayagryllus]) and reversed toward an ancestral state in the taxa which have returned toward an epigean habitat (*Prolonguripies*). Such is the case of a reduced eye size and of a pale body coloration (Fig. 14, 15). The polarity of change for the eye size and the body coloration attributes corroborates the troglobiomorphosis hypothesis.

Conversely, other characters show complex evolutionary transformation series and no simple relation with habitat changes can be found. This is the case of the two attributes used to characterise hindleg size (rat1 and rat2). The phylogenetic patterns derived from cladistic analyses for these attributes showed that neither a shift toward a subterranean habitat nor a reversal toward an epigean life is obligatorily accompanied by a modification of these attribute states, contrary to what is observed for eye size and body coloration. Increase of hindleg size may actually happen in troglobitic taxa, just as it may occur in epigean taxa. In fact no polarity of change seems as-
sociated with habitat modifications, which invalidates the troglobiomor-
phosis hypothesis. As already indicated above, a similar refutation resulted
from the phylogenetic analyses of male forewing and stridulatory apparatus
(Desutter-Grandcolas, 1997d): homoplastic changes (convergences) oc-
curred between epigean and troglobitic taxa for the loss of the stridulum,
and reversals were documented in both epigean and troglobitic taxa.

In the Amphiacustae, the troglobiomorphic hypothesis is thus corrob-
ored by eye size and body coloration, but invalidated by hindleg size and
wing development. Even within a single cricket clade, “troglobiomorphosis”
thus concerns a heterogeneous lot of characters, the state distributions and
evolutionary transformations of which are diverse and contradictory. No
unique pattern of morphological evolution exists. On the contrary, several
patterns are derived from the phylogenetic analyses, involving character state
diversification and homoplasies (convergence or reversal). Such patterns are
commonly documented in phylogenetic reconstructions (Andersen, 1979,
1994; Carpenter, 1989; Coddington, 1990; Wanntorp et al., 1990; Packer,
1991; Desutter-Grandcolas, 1994b, 1997b,d; Andersen and Weir, 1994; Sid-
dall & Burreson, 1995; Grandcolas, 1993, 1996, 1997b; many contributions
in Grandcolas, 1997a) and troglobitic evolution is in this respect not different
from other phyletic evolutions.

So-called troglobiomorphic transformations are often considered adap-
tive to cave life, either directly or through energy economy (Howarth, 1981,
1987; Culver, 1982; Peck, 1990; Kane & Culver, 1992; Culver et al., 1994).
Howarth (1983: 374) thus stated: “Troglolites have evolved to exploit the
organic resources found in mesocavernous and macrocavernous habitats.
The close similarity of cave adaptations among the diverse taxa in so many
different cave areas indicates that cave adaptation is a general process and
the result of similar selection pressures” and precised further: “The adapta-
tions displayed by troglobites include loss or reduction of eyes, cuticular
pigments, wings, a circadian rhythm, and in some species a functional tra-
cheal system; thinning of cuticule; and often the development of longer ap-
pendages, an increase of vestiture, a larger, more slender body form, and in
some species a lower metabolism”. Other hypotheses, such as the accumu-
lation of neutral mutations, pleiotropic effects or genetic drift, have also
been proposed to account for the morphological changes occurring in some
cave dwelling organisms (e.g. Culver, 1982; Howarth, 1987; see also
Wilkens, 1986, 1992; Rouch & Danielopol, 1987; Botosaneanu & Hol-
singer, 1991) and no single hypothesis seems able to account for the avail-
able data (Christiansen, 1992; see also comments in Leroi et al., 1994).

As already indicated, phylogenetic analyses cannot be used to directly
test those process hypotheses, because it is not possible to reasonably assess
that the processes observed in present-day populations are similar to those that were occurring in ancestral populations in ancestral environments (Greene 1986; Leroi et al., 1994). However phylogenetic reconstructions can accurately test for the plausibility of each process hypothesis, by the comparisons of the phylogenetic and predicted patterns: if a process hypothesis implies a given distribution of the states of the character of interest and if this given pattern is different from the pattern reconstructed using phylogeny, then this process hypothesis is refuted in the case under study. This is the most neutral method presently at hand to extend process hypotheses derived from present-day population studies to the past, and to tentatively reconstruct evolutionary pathways: other methods exist, but they are all based on a given set of unwarranted hypotheses which biases and weakens their conclusions (Eldredge & Cracraft, 1980; Grandcolas et al., 1994, 1997). Such a process analysis is far beyond the aim of this paper for lack of data on Amphiacustae populations. Future studies on troglobiomorphy should however integrate both population studies and phylogenetic analyses (taxonomies have long been recognised inadequate references in historical studies) at the scale of monophyletic groups of taxa including both troglobites and epigean organisms, to achieve such phylogenetic tests of process hypotheses.

CONCLUSION

The present paper analyses the troglobiomorphosis hypothesis in one given case study and concludes to a complex situation, for which no simple explanation can a priori be found. Could these results be generalised, and if so how? Obligate cave dwelling organisms are morphologically diverse and distantly related (Vandel, 1964; Howarth, 1983; Juberthie & Decu, 1994). To test for convergent patterns of morphological evolution it would consequently be necessary first to consider separately all the characters that are currently gathered in the “troglobiomorphic syndrome”, second to examine their evolutionary transformations in monophyletic clades including both epigean and troglobitic taxa through phylogenetic analyses, and third to compare by analogy the phylogenetic patterns thus reconstructed. This should allow to propose falsifiable hypotheses concerning the effect of cave living on morphological evolution. By contrast, the troglobiomorphosis hypothesis, as formulated today, may be qualified a “conceptual dead-end”, as it impedes a real test of the hypotheses of troglobitic morphological modifications. Also its current association with habitat characterization biased studies in troglobitic life by imposing ad hoc hypotheses on basic definitions.
Our understanding in cave life has been deeply modified these last thirty years, owing to important discoveries on the subterranean world and organisms (Howarth, 1983; Juberthie, 1984; Juberthie & Decu, 1994). These findings have been easily accepted by Biospeologists and have been followed by a large amount of new, enthusiastic studies.

Now another step in cave life studies is necessary, which concerns the methodology used to analyse troglobitic evolution. Recent increase of knowledge concerning cave life deeply questions traditional ideas on troglobitic evolution. Peck & Finston (1993) rightly observed that point, but stated: “There may not be a unified theory of troglobitic origin, and this may be a general property of the evolutionary biology of caves”. It is clear however that as in any field of Comparative Biology the elaboration of a general theory of cave life origin and cave life evolution, be it with one or several proposals, will result from the independent and reasonable tests of current hypotheses, that is using phylogenetic inference (Eldredge & Cracraft, 1980). Some authors have rightly foreseen this (Deeleman-Rheinhold, 1981; Peck, 1981). Now that a clear methodology in phylogenetic inference is at hand, let us hope that many studies will be carried out in that direction.

ACKNOWLEDGMENTS

I thank M. Bologna and V. Sbordoni for the opportunity to publish this paper, and P. Grandcolas and L. Matile for comments on the manuscripts.

LITERATURE CITED

The Hawaiian cave planthoppers (Homoptera: Fulgoroidea: Cixiidae) - A model for rapid subterranean speciation?

Hannelore Hoch *

SUMMARY

After the successful colonization of a single ancestral species in the Hawaiian Islands, planthoppers of the cixiid genus *Oliarus* underwent intensive adaptive radiation resulting in 80 described endemic species. *Oliarus* habitats range from montane rain forests to dry coastal biotopes and subterranean environments. At least 7 independent evolutionary lines represented by different species have adapted to lava tubes on Molokai (1), Maui (3), and Hawaii Island (3). Behavioral and morphological studies on one of these evolutionary lines on Hawaii Island, the blind, flight- and pigmentless *Oliarus polyphemus* have provided evidence for reproductive isolation between allopatric populations which may in fact be separate species. Significant differences in song parameters were observed even between populations from neighbouring lava tubes, although the planthoppers are capable of underground migration through the voids and cracks of the mesocavernous rock system which is extant in young basalt: after a little more than 20 years, lava tubes within the Mauna Ulu 1974 flow had been colonized by *O. "polyphemus"* individuals, most probably originating from a near-by forest-kipuka. Amazingly, this species complex is found on the youngest of the Hawaiian Islands, with probably less than 0.5 m.y., which suggests rapid speciation processes. Field observations have led to the development of a hypothesis to match underground speciation with the dynamics of vegetational succession on the surface of active volcanoes. Planthopper range partitioning and geographic separation of populations by young lava flows, founder events and small population size may be important factors involved in rapid divergence.

INTRODUCTION

One of the central questions in evolutionary biology is the formation of new species. It has been shown that mating behavior plays a key role in the process of speciation, especially in regard to intraspecific communication (Otte, 1989). To guarantee reproductive success it is essential for conspecific individuals of the different sexes to recognize each other as potential partners. Mobile, sexually reproducing organisms have developed specific be-

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behavioral patterns which serve to bring together males and females of the same species for mating. Each species is characterized by a specific mate recognition system (SMRS) which consists of chemical, optical, tactile and acoustic signals, or a combination thereof (Paterson 1981; 1985). If by any reason the SMRS of two populations diverge to a point where individuals of the different sexes no longer recognize each other as mating partners, i.e., courtship behavior is not initiated and therefore successful matings are no longer possible, these populations are reproductively isolated, and thus have reached the status of biological species.

One of the difficulties one faces when studying the formation of new species in natural populations is that the very process of speciation is gradual and thus withdrawn from direct observation. “Most of the speciational situations with which we deal will already be stabilized, optimized states, with the appropriate evolutionary adjustments having already occurred, so that only the successful systems are available for study. Indeed, it may require a great deal of good fortune to encounter dynamic evolution of a biocommunicative system in natural populations” (Littlejohn, 1988).

A nearly ideal system to study incipient speciation is provided by the cavernicolous insects from Hawaii. Due to its isolated geographic position in the mid of the Pacific, comparatively few plant- and animal species have originally reached the islands (Howarth, 1991). The descendants of those species which successfully colonized the islands in the course of many generations adapted to a large variety of habitats and gave rise to speciose and ecologically diverse lineages: Hawaii is especially well-known for examples of adaptive radiation in flowering plants (e.g., Carr, 1987), surface-dwelling insects (e.g., Hardy, 1974), and birds (Freed et al., 1987). Howarth (1972) demonstrated that adaptive radiation is not restricted to surface habitats by discovering a remarkable rich and diverse obligately cavernicolous (troglobitic) arthropod fauna.

An important element of the Hawaiian cave fauna are cixiid planthoppers of the genus Oliarus. Epigean Oliarus species (Fig. 1) are represented with 80 named taxa (56 species and 24 subspecies) on the main islands, occurring in nearly all vegetation zones where they suck sap from living plants. All Hawaiian Oliarus species are endemic to Hawaii and most likely are descendants of one primary colonizing species. Within Oliarus, at least 7 independent evolutionary lineages have undergone an adaptive shift (Howarth, 1983) to subterranean habitats: 1 on Molokai, 3 on Maui, and 3 on the Island of Hawaii (Hoch and Howarth, unpublished data). One of the lineages that have invaded caves on the Island of Hawaii is Oliarus polyphemus Fennah (Fig. 2), a highly troglomorphic, blind, flight- and pigmentless species. Within the cave ecosystem, O. polyphemus is a rhizophagous primary consumer, sucking sap from
roots of the native tree Metrosideros polymorpha (Myrtaceae) in a perpetually
dark, warm and moisture-saturated environment (Fig. 3). It should be noted
that the primary habitat of this species not only comprises underground spaces
sufficiently large for humans to enter (“caves”), but also an interconnecting
system of voids and cracks (0.1 - 20 cm in width, Howarth, 1983) which is
common in young basalt. Thus, caves are but a window to the mesocavernous
rock system (MSS, *milieu souterrain superficiel*, Juberthie, 1983) where simi-
lar conditions prevail.

Fig. 1 – Epigean *Oliarus* species.

*The SMRS of cavernicolous planthoppers*

Preliminary studies (Howarth et al., 1990) had shown that cavernici-
colous planthoppers – like their epigean relatives – communicate by low-
frequency substrate-borne vibrational signals which are produced by a spe-
cialized organ in the insects’ first two abdominal segments (Ossiannilsson,
1949). In several studies on epigean planthoppers it had been shown that
these signals play a key role in species recognition and are species-specific
(Claridge, 1985).

In cave-dwelling planthoppers, these vibratory signals are pivotal for
the recognition and location of a potential conspecific mate, since in the
cave environment no optical clues are available to the insect, and chemical
communication, e.g., by pheromones is not known in the Fulgoroidea (Hoch and Howarth, 1993). In a field experiment, we demonstrated that living roots are especially well suited for transmission of vibratory signals (Hoch and Howarth, 1993).

Evolutionary dynamics of behavioral divergence among O. polyphemus populations

Previously, O. polyphemus had been regarded a single, widespread species, with morphologically nearly identical populations occurring in similar habitats in numerous lava tubes within Mauna Loa, Mauna Kea, Hualalai and Kilauea volcanic systems. Our study on mating behavior of 7 O. polyphemus populations revealed the following results (Hoch and Howarth, 1993):

1. Communication signals consisted of more or less homogenous pulse trains arranged in time/amplitude-modulated patterns.
2. Variation of call pattern and single call parameters within a population was comparatively low, with male and female calls resembling each other.
3. Variation of call pattern and single call parameters between populations was high in both sexes, in some cases even differing significantly.
4. Playback-experiments using individuals from different populations provided evidence that individuals of a given population responded prefe-
Fig. 3 – Interior of a lava tube with root curtains of *Metrosideros polymorpha*. 
rably to an opposite-sex individual from that same population, whereas individuals from different populations did apparently not recognize each other as potential mates, and courtship behavior was not initiated.

These results suggest that *O. polyphemus* has to be regarded not as a single, widespread species but rather as a complex of morphologically similar and most probably closely related, yet separate, i.e. reproductively isolated, species. Since so far only a few populations were studied, it is completely unclear of how many biological species “*O. polyphemus*” consists. It is remarkable that even between populations from caves which are only a few kilometers apart, there is no indication of genetic exchange – this would be easily conceivable considering the possibilities of underground migration of these small insects via the mesocavernous rock system.

*Does active volcanism promote speciation in obligately cavernicous planthoppers?*

How can we explain the observed divergence of a cavernicous organism on one island within a comparatively short span of time? According to recent estimates, Hawaii Island is less than 400,000 years old. Keeping in mind that this oceanic island had to be colonized by higher plants first to support subsequently colonizing insects species, we can assume that time available for the evolution of cave-adapted species was even much less than that. A key to understanding of the evolutionary processes which are underlying the observed pattern may be found in the geology and vegetation dynamics on active volcanoes. Recent radiocarbon-dating of ash layers and lava flows of Mauna Loa have shown that the surface of this volcano is renewed at a rate of ca. 40% in 1,000 years (Holcomb, 1987; Lockwood and Lipman, 1987). For Kilauea, today the most active volcano on Hawaii Island, these estimates may even be too low. Own field studies have shown that the primary habitat of *O. “polyphemus”* is characterized by a specific stage in the succession of surface vegetation: in the field, this stage is characterized by sparse vegetation cover consisting of species pioneering on new lava flows, e.g., *Sadleria* and *Cibotium* ferns, and *Metrosideros* (Fig. 4). This stage is intermediate in the succession of vegetation on new lava flows: from completely unvegetated areas to the formation of a dense forest cover. This “intermediate” stage of vegetational succession provides ideal conditions for cave planthoppers: on the surface, the still sparsely vegetated flow is comparatively dry and soil development not far progressed. Colonizing plants thus produce large quantities of long roots which penetrate the porous rock to utilize percolating water. Lava tubes underneath these formations usually house large *Oliarius* populations. Over time,
Fig. 4 – *Metrosideros polymorpha* colonizing a young lava field.
progressive succession and erosion lead to the development of a soil layer, which will eventually support a dense forest cover. In these areas, roots are concentrating inside the absorbing soil layer. Field observations revealed that lava tubes underneath densely vegetated flows usually show sparse root development and consequently, *O. "polyphemus"* individuals are rarely seen. On active volcanoes, the described process of vegetational succession is repeated continuously as new lava destroys established vegetation on the surface (and the roots underneath), but also provides soon (within few years) new substrate for pioneering plants and consequently, new habitat for cavernicolous *Oliarus*. The dynamics of vegetational succession on the surface is closely correlated with the destruction and creation of planthopper habitat: fragmentation of populations by new lava and repeated colonization of newly available areas with adequate resources are likely to be the driving forces of diversification among *O. "polyphemus"* populations. Field observations corroborate these assumptions: in 1995, we discovered *O. "polyphemus"* individuals (nymphs and adults) in a lava tube within a 1974 lava flow of Mauna Ulu. The margin of the nearest adjacent vegetated area being about 200 m away, the surface about the cave was still very sparsely colonized by ferns and *Metrosideros* (Fig. 5). Thus, we can assume underground migration at a rate of ca. 10 m/year which corresponds with laboratory observations.

Fig. 5 – Lava field of Mauna Ulu: 1974 eruption.
Fig. 6 – Hypothetic distribution of cave planthoppers and dynamics of their habitat in the course of vegetational succession on the surface. A. Grey-shaded area: distribution of suitable habitat; dash lines: range of a hypothetical rhizophageous cavernicolous species. B. Range fragmentation of ancestral population by new lava flows. C. Migration of cave planthoppers (arrows) following the distribution of their habitat: from areas under forest cover (black dots) to areas underneath adequately vegetated lava flows.
It is conceivable that founder effects, small population size, and/or sexual selection facilitate rapid manifestation of differentiation (e.g., in regard to SMRS) acquired during separation. Upon secondary contact, these differentiations may act as factors in reproductive isolation. In the laboratory, secondary contact was simulated by playback experiments; in the field we have indications for sympatric occurrence of two O. "polyphemus" species from only one lava tube (Pink Pistillaria Cave, Hualalai volcano) (Hoch and Howarth, 1993).

The hypothesis that active volcanism promotes speciation processes in cavernicoles is supported by observations on other islands in the Hawaiian chain: Maui and Molokai, both also comparatively young – and until quite recently volcanically active – islands, house cave planthopper species. On Oahu and Kauai, the geologically oldest of the “high” islands of Hawaii, erosion is far more progressed: accordingly, the extent of suitable habitat is much smaller, and no cave planthoppers have been found.

To test this hypothesis and to gain deeper insight in the mechanisms involved in incipient speciation, a population genetic study focusing on the phylogenetic relationship among select O. "polyphemus" taxa from various lava tubes is under way at the University of Missouri (K. Williamson). It is hoped that by using adequate genetic markers, we will learn to understand the evolutionary history of Oliarus. The information from the genetic studies can also be used to calibrate speciation events against geologic information, i.e., to determine evolutionary time.

ACKNOWLEDGMENTS

I would like to express my sincere thanks to Dr. Manfred Asche, Museum für Naturkunde, Berlin, for his support, his constructive criticism, and his never-fading enthusiasm in the field which kept me going through the tightest crawls. I also thank Dr. Frank Howarth, Bishop Museum, Honolulu, for fruitful discussions, continued stimulation and for his good humour which has highlighted many of our common field trips. Dr. Manfred Ade, Museum für Naturkunde, Berlin, kindly helped with image processing of figure 6. Last but not least I give a big mahalo to all the people in Hawaii who have in many ways contributed to this study, e.g. the staff of Bishop Museum, the University of Hawaii at Manoa and Hilo, The Nature Conservancy, Hawaii Volcanoes National Park, and private landowners for their support, their generous sharing of information and their trust that we might use it to help preserve one of Hawaii’s most intriguing ecosystems.

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Climatic fluctuations and tropical troglobitic evolution

Pedro Gnaspini *

Allopatric speciation (which is considered to be the most probable model of speciation in general) of cave organisms requires geographic isolation as a result for instance of local extinction of epigean populations. A classical model proposed to explain the origin of terrestrial troglobites in temperate zones (e.g., Barr, 1968; Culver, 1982) relates this local extinction to long-term climatic fluctuations, such as Pleistocene glacial cycles. From this model, it is predicted that: 1) Regions subject to more frequent and accentuated fluctuations will present a higher ratio troglobitic/troglophilic species, especially in the case of terrestrial fauna. 2) Dry, arid or semi-arid areas will bear the most specialized troglobites.

This model can be applied to the origin of tropical troglobites as well, especially for terrestrial organisms. Evidences of Quaternary climatic changes in tropical South America are indisputable, indicating drier climates related to glacial periods (Figs. 1 and 2). Moreover, preliminary comparative data on the composition of Brazilian cave fauna seems to fit those predictions (for discussion, see: Gnaspini & Trajano, 1994; Trajano, 1995).

For instance, relatively few terrestrial troglobites are found in those areas in Central Brazil (n° 1 in Figs. 1, 2) which remained covered by a cerrado vegetation similar to that of the present time during the last glacial period.

Highly specialized troglobites and some "relictual" hygrophilic organisms (for instance, see Gnaspini et al., 1998) have been found in caves of northeastern Brazil (n° 2 in Figs. 1, 2) (and in the Argentinian Patagonia as well – see Trajano, 1991). These animals may have colonized the subterranean biotope during the short periods of higher humidity (possibly near the interglacial maxima) and became isolated for long periods since then (including the glacial periods and part of the interglacials).

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Less specialized troglobites are found in areas now covered by humid vegetation but that had dried during glacial periods, as seems to be the case of the southeastern Brazil (n° 3 in Figs. 1, 2). It probably happened because the total time of isolation is shorter (all or part of the glacial). Unfortunately, there are some areas in Brazil which are still scarcely surveyed from the biospeleological point of view (such as numbers 1 and 2 in Figs. 1, 2), when compared with southeastern Brazil (n° 3 in Figs. 1, 2). A more intense study of the latter areas (and of other tropical areas as well) will probably result in a larger number of highly specialized tropical troglobites and more data to corroborate the hypotheses discussed herein.

Fig. 1 - Present morphoclimatic domains in Brazil (modified from Ab’Saber, 1977a). The Speleological Provinces (modified from Trajano & Sanchez, 1994) are placed in the same map. (1) = Bodoquena Ridge Speleological Province; (2) = Bambui Speleological Province; (3) = Ribeira Valley Speleological Province.
Fig. 2 – Past (between 13,000 and 18,000 years b.p.) morphoclimatic domains in Brazil (modified from Ab’Saber, 1977b). The Speleological Provinces (modified from Trajano & Sanchez, 1994) are placed in the same map. (1) = Bodoquena Ridge Speleological Province; (2) = Bambui Speleological Province; (3) = Ribeira Valley Speleological Province.

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A simple scenario for stygobitization in *Stenobermuda* Schultz, 1978 (Isopoda Asellota Stenetriidae), with description of a new species from Andros Island, Bahamas

Lazare Botosaneanu * and Thomas M. Iliffe **

**SUMMARY**

Description of a new stygobitic and troglomorphic species of *Stenobermuda* from a Blue Hole in the Bahamas, is an opportunity for speculation about hypogean colonization by this and by another cave-dwelling species from Bermuda, starting from populations of a widely distributed Western Atlantic shallow water marine species.

**INTRODUCTION**

Gnathostenetroidoidea Kussakin, 1967, and Stenetiloidea Hansen, 1905, are two of the four superfamilies in which Isopoda: Asellota are presently divided. These two superfamilies are considered as being the most primitive Asellota.

Despite much progress achieved in study of diversity in the two superfamilies, incertitude still reigns concerning their characterization and delimitation, the generic limits and the relationships between genera, and even on author names conflicting opinions were published (most of the papers in the References, and several more, would have to be quoted here in support of this statement). For example, genus *Stenobermuda* was described in Stenetriidae (Schultz, 1978) but transferred to Gnathostenetroididae by Kensley (1994). In our opinion, the male pleopodal arrangement in species of this genus matches better the pattern shown for Stenetrioidea in Wägele (1983: Fig. 4) and in Kensley & Schotte (1989: Fig. 34 - and not the diagnoses in this last publication, which sometimes are at strong variance with this figure), and we maintain *Stenobermuda* in Stenetriidae. Much evidence from the revision of Stenetriidae by Serov & Wilson (1995) clearly supports this decision.

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Representatives of the two superfamilies are in their overwhelming majority marine, with the centre of gravity in shallow waters. Quite a few are known to have colonized subterranean waters in karst or in porous habitats, and in some cases the Crenal (listed, with various information, in Henry, Lewis & Magniez, 1986; subsequently described were: *Stenobermuda iliffei* Kensley, 1994; *Caecostenetroides ruderalis* Stock & Vonk, 1990; and *C. ascensionis* Vonk & Stock, 1991).

The present paper is a contribution to the knowledge of stygobitization in primitive Asellota (see Conclusions).

*Stenobermuda mergens* n.sp.

Figs. 1-9

**Material and locality**

Male holotype from Conch Sound Blue Hole, Andros Island, Bahamas. Collected by diving (6.IV.1996, by Brian Kakuk) with plancton net in 26 m water depths at 750 m penetration into the cave. A series of appendages missing or broken. Were dissected for study only those appendages strictly necessary for describing the new species (dissecting more would have resulted in completely destroying the unique available specimen). Deposited in the Zoological Museum of the University of Amsterdam.

**Description of male**

Length (from tip of rostrum to end of pleotelson): 3.2 mm. Completely depigmented. Eyes (ommatidia) absent. Ratio body length/maximal width (being that of pereionites II and III) slightly exceeding 3.5.

Distal margin of cephalon with triangular, not very acute, rostrum; frontal processes attenuate, anterolateral processes acute, well developed but much shorter than rostrum. The longest pereionites are II and III; pereionites I-III with anterolateral corners pointed and directed anteriad; pereionite IV with small obtuse anterolateral corners followed posteriad by a distinct emargination; pereionites V-VI laterally very obtuse; pereionite VII with acute posterolateral corners directed posteriad. Two very small free pleonites. Pleotelson like in the two already described *Stenobermuda* (but also like in some *Stenetrium*), relatively broad, distal margin slightly sinuous, moderately produced (obtuse). Uropods missing.

Maxillipedal endite with only two very characteristic coupling hooks looking like crosses, on its straight median margin; distal margin with 7
spines, all simple, arranged like in Fig. 3. Maxillipedal palp typical for *Stenobermuda*.

Carpus of gnathopod with row of some 10 strong setae on internal margin. Propodus distally strongly widening (distal margin only slightly shorter than internal margin, and with a hyaline "blade" along most of its length); on distal margin ("cutting edge") 7 spines - that in the internal corner by far the strongest--; on internal margin (palm) 5 spines, all spines finely pectinate. Pereiopod II (and following): propodus ending in strong conical projection on which a pair of fine setae are inserted.

Pleopods I with very small (i.e.: short and narrow) common protopodite; they form an operculum covering pleopods II and have relatively numerous short setae along their lateral margins.

Fig. 1 – *Stenobermuda mergens* n.sp., male holotype: habitus.
Figs. 2-4 – *Stenobermuda mergens* n.sp., male holotype: left maxilliped, without the epipodite; distal margin of its endite; and coupling hooks (3 and 4 more strongly magnified than 2).

The very small pleopods II with uniarticulate exopodite ("a" in Fig. 7) having the shape of a parallelogram, slightly emarginate distally, with long subapical seta and serrate internal margin. Copulatory endopodite biarticulate, strongly flexed, 1st article swollen, 2nd article of very complex structure: anteapical part ("b") widened and bilobed, apically with two appendages: lower one ("c") a darker, slender "spine" with shorter "tooth" near its root, and with apex surrounded by a hyaline blade; upper one ("d") broadly oval, with small, hyaline, wrinkled "crown".

Pleopods III: endopodite much more slender than the biarticulate exopodite, tapering towards the truncate apex on which four plumose setae are inserted; both articles of exopodite strongly widened.
Comparisons

The new species clearly belongs to genus *Stenobermuda* Schultz, 1978, in which two species were already described: *S. acutirostrata* Schultz, 1978, and *S. iliffei* Kensley, 1994 (according to Kensley, 1994 "It is probable that several species described under *Stenetrium* are actually representatives of *Stenobermuda*"; and in Serov & Wilson, 1995, the South African marine littoral *Stenetrium syzygus* Barnard, 1940, is transferred to *Stenobermuda*). In what follows we shall mention only characters for which comparison with the published descriptions and illustrations shows clear differences.

Figs. 5-6 – *Stenobermuda mergens* n.sp., male holotype: left gnathopod from merus on, and apical part of pereiopod II (6 more strongly magnified than 5).
From *S. acutirostrata* the new species differs in: the smaller size; the complete depigmentation (however, in the original description of *acutirostrata* we read “Pigmentation light if at all”); the anophtalmy; a less pointed rostrum and shorter anterolateral processes of cephalon; the blunt lateral ends of pereionites IV-V (and possibly other details of the pereionites); the relatively broad and distally less produced pleotelson; the very distinctive coupling hooks of the maxilliped endite; the uniarticular exopodite of pleopod II (this is illustrated – but not described – as biarticulate in *acutirostrata*), as well as details of its endopodite (although – compare our Fig. 8 with Fig. 3 in Schultz, 1978 – there seems to be some similarity in the structure of its apical appendages).
From *S. iliffei*, *S. mergens* n.sp. can be distinguished by: the anophtalmy; the very distinctive coupling hooks of the maxillipedal endite, and the very different armature on its distal margin; the distinctly more strongly widened gnathopod propodus and the more numerous strong setae on the internal margin of its carpus; the strong conical projection at the apex of pereiopod II (and following) propodus; the smaller common protopodite of pleopods I; the shape of the exopodite of pleopod II (however, a similarity seems to be the fact that also in *S. iliffei* the exopodite seems to be uniarticulate: Fig. 10 C in Kensley, 1994); the abundantly different structure of the two apical appendages of pleopod II endopodite.

*Derivatio nominis*

The specific name was coined from (Lat.) mergo = to dive, alluding to the fact that the specimen, caught by diving, belongs to a species “diving” in the depths of Blue Holes.
CONCLUSIONS

_**Stenobermuda acutirostrata**, S. iliffei, and S. mergens n.sp. are doubtless closely related species. **S. acutirostrata** was described (Schultz, 1978) from the South shore of Bermuda, from “a bottom of sand and rocks at 90m”. It was rediscovered (Schotte, Heard & Kensley, 1991) in two localities in the Caicos Islands: “Rack Cay, sponge washings” and “Pine Cay, inside fringing reef, rubble- sand substratum, 4-5m”. Meagre as it is, the available evidence shows that this is a shallow water species widely distributed in the Western Atlantic. It is lightly pigmented and has apparently eyes consisting of some 5 ommatidia. **S. iliffei** is known (Kensley, 1994) from Walsingham Cave, Bermuda, where – judging from the number of collected specimens – certainly an important population is present; the species is oculate (eyes consisting of only 4 ommatidia) and probably a relatively recent subterranean colonist. **S. mergens** n.sp., completely depigmented and anophtalmous, was collected from the depths of a Blue Hole of Andros Island, Bahamas. It is practically certain that **S. iliffei** and **S. mergens** are hypogean offshoots of different populations of **S. acutirostrata**; we predict that other populations of these two species, and possibly also other closely related subterranean-adapted species will be discovered in hypogean habitats inside the distribution area of the marine species. The colonization of subterranean aquatic biotopes by shallow water marine elements in Gnathostenetroidoidea and Stenetrioidea is considered (Wägele, 1990) as being a general phenomenon.

The stygobiological literature abounds in examples supported by serious evidence (from morphology, distribution, and ecology) of several closely related stygobitic/trogloomorphic species being apparently offshoots – of synchronic or asynchronic origin – from different populations of a recent epigean species. Cladists could to their profit ponder over this matter; the existence of such “bushes” contradicts a central dogma of cladistics: the universality of dichotomous branching as phylogenetical scenario (Hoelzer & Melnick, 1994; Kolibác, 1997).

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Geographic variation and genetic relationships in populations of the *Androniscus dentiger* complex from Central Italy (Isopoda, Oniscidea, Trichoniscidae)

Gabriele Gentile and Giuliana Allegrucci *

**SUMMARY**

*Androniscus dentiger* is a terrestrial isopod distributed from Great Britain to North Africa, inhabiting humid edafic environments, superficial underground compartments and both natural and artificial caves. In this study allozyme data have been used to investigate the geographic variation and the genetic relationships of several populations of *A. dentiger* from Central Italy, using as outgroups populations from four congeneric species, *A. calcivagus, A. cfr. subterraneus, A. spelaeorum,* and *A. degener.* Multivariate analysis of *A. dentiger* allele frequencies indicates the existence of a group of populations (group A) distributed in a wide geographic area which are genetically slightly differentiated, and several populations (arbitrarily defined as group B) which show differentiation levels comparable to those observed between the morphologically well differentiated species. The low valley of the river Tiber seems to act as an effective geographic barrier between the populations from group A and the remaining ones. The genetic divergence between populations within the group A seems to have a recent origin. This is suggested by the low genetic distances and heterozygosity values within the group A, and by the very low number of private alleles occurring in this group. The high degree of intraspecific and interspecific genetic differentiation is not consistent with the levels of morphological differentiation traditionally used to distinguish different species within this genus. On the whole, these data suggest that *A. dentiger* might be considered as a complex of cryptic/sibling species.

**INTRODUCTION**

The terrestrial isopod *Androniscus dentiger* inhabits, as other congeneric species, humid edafic environments, superficial underground compartments, and both natural and artificial caves. Usually, in Trichoniscidae, highly hygrophilic habits represent a strong constraint for dispersal. Evidence of this phenomenon is the high number of taxa (both at the species and genus level) which are geographically differentiated, and are also narrow endemics. *A. dentiger,* unlike other congeneric species and other Trichoniscidae, is widely distributed. It occurs in Great Britain, Central Europe, mainland Italy, Sicily, and North Africa and its range has been considered to be in a phase of active and passive (by man) expansion (Van-
del, 1960). However, *A. dentiger* does not occur in Corsica (Taiti and Ferrara, 1996), Tuscanian archipelagos and in some localities along the Tuscanian coast (Taiti and Ferrara, 1980) where potentially colonizable environments occur. Moreover, this species is missing in many suitable caves within its range (Gentile, unpublished data).

In a previous study the levels of gene flow among populations of *A. dentiger* in Central Italy have been investigated using different *Nm* estimators. The very low levels of gene flow reported, even between neighboring populations, suggested that nearly all the populations studied have isolated gene pools (Gentile and Sbordoni, 1998). In this paper we discuss the geographic variation and the genetic relationships among the same *A. dentiger* populations, using as outgroups populations from four species belonging to the same genus.

**MATERIAL AND METHODS**

Twenty eight populations of *A. dentiger* from Central Italy have been studied, including cave and surface populations. Six populations taxonomically assigned to four different species (*A. calcivagus, A. crf. subterraneus, A. spelaeorum* and *A. degener*) were used as outgroups. These species are morphologically well differentiated (Vandel, 1960), and occur only in the North-Eastern Italian Prealpine mountains. Some of these species could also be found in syntopy. In these cases, no evidence of hybridization could be highlighted. In Table 1 the populations studied are reported. Cave and surface populations are indicated with a three letter symbol in upper and lower cases types, respectively.

Genetic variation was investigated using allozyme electrophoresis on cellulose acetate gels. The following enzymes were assayed: *Ada, Aldo, Ca, Dia, Me, Aph, Pgm, BGal, G6pd, Gpi, Got, Idh, Mdh, Mpi, Pep, Pk*, for overall 19 gene loci scored. Details of the protocols used and allele frequencies are reported in Gentile and Sbordoni (1998).

Heterozygosity, Nei’s (1972) and Reynolds’ (Reynolds et. al., 1983) genetic distances were calculated using *GEN-SURVEY* (Vekemans and Lefebvre, 1997). We used the Nei’s and Reynolds’ indexes to provide more accurate dating of events of divergence in different evolutionary contexts. We used Nei’s (1975) relationship *t=kd*, where *t* is the time of divergence, *k* (the substitution rate) is equal to 5*10^6 and *D* is the Nei’s distance. Reynolds’ index, which assumes divergence to be caused only by genetic drift, was used in a context of short-term evolution. We applied the formula *t≈D/2N*, where *t* is the time of divergence, *N* the effective population size and *D* is the Reynolds’ coefficient. We estimated an average population size ranging from 500 to 5,000 individuals.

Table 1 – Sample sites
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<th>Species</th>
<th>Locality</th>
<th>Toponym</th>
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<th>Latitude</th>
<th>Longitude</th>
<th>Temp. ('C)</th>
<th>Label</th>
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<td>Grotta di Nerone</td>
<td>1025</td>
<td>43°34'16&quot;</td>
<td>12°30'39&quot;</td>
<td>7,1</td>
<td>NER</td>
</tr>
<tr>
<td></td>
<td>Castelnuovodl Garlagnana (LU)</td>
<td>Tanadi Magnano</td>
<td>630</td>
<td>43°10'36&quot;</td>
<td>10°23'09&quot;</td>
<td>12,7</td>
<td>val</td>
</tr>
<tr>
<td></td>
<td>Piteglio, S. Marcello Pistoiese (PT)</td>
<td>Lana Termini</td>
<td>349</td>
<td>44°02'27&quot;</td>
<td>10°43'17&quot;</td>
<td>11,8</td>
<td>TER</td>
</tr>
<tr>
<td></td>
<td>Tos (FI)</td>
<td>Buca delle Fate</td>
<td>465</td>
<td>43°46'00&quot;</td>
<td>11°27'00&quot;</td>
<td>11,5</td>
<td>TOS</td>
</tr>
<tr>
<td></td>
<td>Livorno Montenaro (LU)</td>
<td>Cava del Santuario</td>
<td>193</td>
<td>43°29'00&quot;</td>
<td>10°21'00&quot;</td>
<td>14,5</td>
<td>mon</td>
</tr>
<tr>
<td></td>
<td>Piobbio, Monte Nerone (PS)</td>
<td>Grotta di Nerone</td>
<td>1025</td>
<td>43°34'16&quot;</td>
<td>12°30'39&quot;</td>
<td>7,1</td>
<td>NER</td>
</tr>
</tbody>
</table>

Statistical significance of heterozygosity estimates and genetic distances between and within groups of populations was tested by 1000 bootstrap cycles over populations (Van Rossum et al., 1997; Vekemans and LeFebvre, 1997). The program GENETIX ver. 3.0 (Belkhir et al., 1996) was used to test the null hypothesis $D=0$ for each pair of populations.

An ordination of *A. dentiger* populations was carried out by means of the Factorial Correspondence Analysis carried out on allele frequencies (FCA, Benzecri et al., 1973). A geographic contour map was obtained by interpolating the scores of the first axis of the Factorial Correspondence Analysis (Cavalli-Sforza et al., 1994).

The neighbor-joining (NJ tree, Saitou and Nei, 1987) method was applied to a matrix of genetic distances (Nei, 1972). Robustness of each node was evaluated by bootstrapping allele frequencies 1000 times, using the program SEQBOOT in PHYLIP 3.57 (Felsenstein, 1995).

We also carried out parsimony analyses on allozyme data. Allozymes were recoded considering a locus as a character, and a combination of alleles occurring at that locus as a state (Mabee and Humphries, 1993). In-
stead of ordering the character states and imposing a specific pathway, we considered all transformations to be possible. In a stepmatrix, a cost to every possible transformation was assigned by assuming that each gain or loss of an allele equals one evolutionary step. We used ASAP 1.5 (Thumfort and Sampson, personal communication) to recode allozyme data according to the procedure assessed in Mardulyn and Pasteels (1994). Most-parsimonious (MP) trees were derived by the heuristic search as implemented in PAUP 3.1.1 (Swofford, 1993). Ten random replicates of a heuristic search were performed. The options random and tree-bisection-reconnection (TBR) were used for stepwise addition and branch swapping procedures, respectively. The MP tree and the shortest trees supporting alternative phylogenetic hypotheses were compared using Templeton’s (1983) test, as detailed in Larson (1994).

RESULTS

Figure 1 shows the results of the Factorial Correspondence Analysis carried out on allele frequencies of the 28 populations of A. dentiger. The first axis which explained 24.5% of variance allowed the discrimination between two major groups: group A, including populations distributed in a wide area ranging from the Apennines of Tuscany and Marches to the alluvial plains of Tuscany and Latium, and the group B including the remaining populations. The second axis (13%) clearly separates STI and PIA populations from all the others, while the third axis (10.2%) discriminated populations DVL, PIA, SUB and CHI. The second and third axis indicated that the Apennines populations do not form an homogeneous group.

Alternative alleles and a high number of private alleles occurred in most loci. In the Figure 2 the percentage of alleles which are shared by an increasing number of populations (represented by histograms) is reported together with their average frequency (represented by line). More than 40% of all alleles scored are shared by a maximum of three populations. These alleles showed an average frequency equal to 0.4.

Only two private alleles occurred in the group A, while 11 private alleles were found in the group B. Nearly all the alleles shared only by two and three populations were in group B.

Mean heterozygosity per population is reported in Table 2. Average heterozygosity estimates between and within groups A and B are reported in Tables 3a and 3b. We did not observe a statistically significant difference in mean heterozygosity between cave and surface populations. However, if groups A and B were analyzed separately, heterozygosity levels were statistically different between cave and surface populations within the group B.
Nei's genetic distances are reported in the Appendix. Genetic distances between and within groups A and B are summarized in Tables 3 and 4. Within A. dentiger intraspecific genetic distance values between populations are generally high, with an average of 0.493 ± 0.013. The genetic distance between group A and group B is very high (D=0.670; p=0.000).

Interspecific distances ranged from 0.3 to 1.539, with a mean of 0.749 ± 0.019. A. cfr. subterraneus has the smallest distance value from A.dentiger (D=0.578±0.027), whereas A. degener has the highest one (D=1.093±0.040). Average D values from A. dentiger and the two remaining species, A. calcivagus and A. spelaearom, are 0.680±0.020 and 0.683±0.023, respectively.
The topology of the NJ tree obtained is shown in Fig. 3a. The tree is arbitrarily rooted at A. degener (VJ1), the most differentiated species. A. dentiger splits into the groups A and B, being divided by A. calcivagus. A. calcivagus is the only robust cluster of the whole tree (bootstrap values >70%). The remaining two species, A. cfr. subterraneus and A. spelaeorum, link together and are nested with group B.

Table 2 – Genetic variability at 19 loci in the 34 populations of Androniscus.

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean no. of alleles per locus</th>
<th>Percentage of loci polymorphic(*)</th>
<th>Mean heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>Androniscus dentiger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPI</td>
<td>1.6 ± 0.1</td>
<td>26.3</td>
<td>0.069 ± 0.024</td>
</tr>
<tr>
<td>MAG</td>
<td>1.7 ± 0.1</td>
<td>42.1</td>
<td>0.123 ± 0.034</td>
</tr>
<tr>
<td>TER</td>
<td>1.7 ± 0.1</td>
<td>26.3</td>
<td>0.147 ± 0.051</td>
</tr>
<tr>
<td>can</td>
<td>1.4 ± 0.1</td>
<td>21.1</td>
<td>0.087 ± 0.035</td>
</tr>
<tr>
<td>ONF</td>
<td>1.3 ± 0.1</td>
<td>15.8</td>
<td>0.072 ± 0.039</td>
</tr>
<tr>
<td>TOS</td>
<td>1.5 ± 0.1</td>
<td>26.3</td>
<td>0.084 ± 0.032</td>
</tr>
<tr>
<td>non</td>
<td>1.5 ± 0.1</td>
<td>36.8</td>
<td>0.100 ± 0.037</td>
</tr>
<tr>
<td>NER</td>
<td>1.6 ± 0.1</td>
<td>36.8</td>
<td>0.101 ± 0.037</td>
</tr>
<tr>
<td>val</td>
<td>1.4 ± 0.1</td>
<td>42.1</td>
<td>0.094 ± 0.030</td>
</tr>
<tr>
<td>tec</td>
<td>1.5 ± 0.1</td>
<td>36.8</td>
<td>0.101 ± 0.032</td>
</tr>
<tr>
<td>MEZ</td>
<td>1.5 ± 0.1</td>
<td>26.3</td>
<td>0.088 ± 0.032</td>
</tr>
<tr>
<td>DVL</td>
<td>1.4 ± 0.1</td>
<td>15.8</td>
<td>0.069 ± 0.030</td>
</tr>
<tr>
<td>TOM</td>
<td>1.2 ± 0.1</td>
<td>15.8</td>
<td>0.062 ± 0.035</td>
</tr>
<tr>
<td>SUB</td>
<td>1.7 ± 0.2</td>
<td>52.6</td>
<td>0.158 ± 0.043</td>
</tr>
<tr>
<td>ORS</td>
<td>1.5 ± 0.1</td>
<td>31.6</td>
<td>0.089 ± 0.028</td>
</tr>
<tr>
<td>RIP</td>
<td>1.5 ± 0.1</td>
<td>42.1</td>
<td>0.130 ± 0.037</td>
</tr>
<tr>
<td>CHI</td>
<td>1.3 ± 0.1</td>
<td>21.1</td>
<td>0.072 ± 0.040</td>
</tr>
<tr>
<td>vel</td>
<td>1.9 ± 0.2</td>
<td>47.4</td>
<td>0.169 ± 0.042</td>
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<tr>
<td>vig</td>
<td>1.5 ± 0.1</td>
<td>31.6</td>
<td>0.062 ± 0.018</td>
</tr>
<tr>
<td>mal</td>
<td>1.5 ± 0.1</td>
<td>36.8</td>
<td>0.125 ± 0.039</td>
</tr>
<tr>
<td>STI</td>
<td>1.3 ± 0.1</td>
<td>10.5</td>
<td>0.029 ± 0.018</td>
</tr>
<tr>
<td>PIL</td>
<td>1.5 ± 0.1</td>
<td>26.3</td>
<td>0.062 ± 0.021</td>
</tr>
<tr>
<td>var</td>
<td>1.7 ± 0.2</td>
<td>26.3</td>
<td>0.115 ± 0.040</td>
</tr>
<tr>
<td>pop</td>
<td>1.5 ± 0.1</td>
<td>21.1</td>
<td>0.108 ± 0.044</td>
</tr>
<tr>
<td>tua</td>
<td>1.6 ± 0.2</td>
<td>36.8</td>
<td>0.118 ± 0.040</td>
</tr>
<tr>
<td>ant</td>
<td>1.7 ± 0.2</td>
<td>36.8</td>
<td>0.141 ± 0.043</td>
</tr>
<tr>
<td>TRV</td>
<td>1.6 ± 0.2</td>
<td>36.8</td>
<td>0.120 ± 0.041</td>
</tr>
<tr>
<td>Androniscus calcivagus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOT</td>
<td>1.5 ± 0.1</td>
<td>21.1</td>
<td>0.084 ± 0.040</td>
</tr>
<tr>
<td>MIN</td>
<td>2.0 ± 0.2</td>
<td>47.4</td>
<td>0.151 ± 0.036</td>
</tr>
<tr>
<td>LAG</td>
<td>1.6 ± 0.1</td>
<td>42.1</td>
<td>0.125 ± 0.038</td>
</tr>
<tr>
<td>Androniscus cfr. subterraneus</td>
<td>1.4 ± 0.1</td>
<td>21.1</td>
<td>0.051 ± 0.021</td>
</tr>
<tr>
<td>VJ2</td>
<td>1.4 ± 0.1</td>
<td>21.1</td>
<td>0.051 ± 0.021</td>
</tr>
<tr>
<td>Androniscus spelaeorum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POL</td>
<td>1.4 ± 0.1</td>
<td>26.3</td>
<td>0.095 ± 0.040</td>
</tr>
<tr>
<td>Androniscus degener</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VJ1</td>
<td>1.5 ± 0.1</td>
<td>31.6</td>
<td>0.066 ± 0.020</td>
</tr>
</tbody>
</table>

(*) A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95
ANDRONISCUS DENTIGER COMPLEX FROM CENTRAL ITALY

The parsimony analysis produced 112 equally parsimonious trees (length=252). The MP tree is reported in Figure 3b. It is also rooted at A. degener (VJI), and shows that A. dentiger splits into two different groups of populations, mainly corresponding to the groups A and B obtained by FCA, and the NJ tree. Groups A (plus SPI) and B are separated by the insertion of A. calcivagus and A. spelaeorum. A. cfr. subterraneus is nested with group B. We used Templeton’s (1983) test to determine whether we could reject the hypothesis of the monophyletic origin of A. dentiger. We compared the MP tree to the most parsimonious tree obtained by forcing the monophyly of A. dentiger. The tree with A. dentiger monophyletic required 5 steps more than the MP tree. However, it was not significantly different from the MP tree.

DISCUSSION

Geographic variation and evolutionary patterns

Multivariate analysis (fca) of allele frequencies (Fig. 1) pointed out the existence of at least two groups of populations. The populations belonging to group A are strongly divergent from the remaining ones (group B). In the
South, the low valley of the river Tiber divides the two groups and might have represented a notable barrier to gene flow. Interestingly, the Tiber valley also represents a geographical barrier between populations belonging to the cave crickets *Dolichopoda laetitiae-geniculata* complex (Cesaroni et al., 1997).

Groups A and B show different geographical distribution and even very different evolutionary patterns. In group A the number of private alleles is low, suggesting that mutation did not play an important role in the evolutionary process within this group. Average genetic distances (Nei’s index) within group A (Tables 3a,b; 4) suggest that the times of divergence between most of the *A. dentiger* populations within group A are very recent. The Reynolds’ coefficient relates the divergence times within group A to a time-span ranging between 17,000 and 1,700 years ago. This estimate would correspond to the wide expansion in Central Italy of the mesophilic forests, which represent the main routes for dispersal of *A. dentiger*. This expansion started at the beginning of the Holocene (14,000 years ago) until the present (Magri and Follieri, 1992), after a long period (300,000 years) when mesophilic forest environments occurred in very few and short periods, and were limited to very narrow areas (Follieri et al., 1993). This scenario may explain the absence of *A. dentiger* in the whole of the tuscian archipelagos and in the tuscian coastal mountains (Taiti and Ferrara, 1980), which were connected with the mainland only in the last 10,000 years (Lanza, 1984).

Figure 4 shows a geographic representation of the genetic variation observed. The darkest area groups the most similar populations belonging to group A. It might be the possible area from which propagules from a limited number of populations started the colonization of the alluvial lands of Tuscany. Genetic drift might be responsible for the decreased genetic variability within new populations, where alleles which are rare in the source populations are less likely to be represented. Consistently with the hypothesis of a recent colonization, average genetic distance among surface populations within group A is comparable with the value obtained for cave ones (Tables 3a,b).

Mutation seems to be one of the main factors shaping the evolutionary pattern within group B. In this group in fact, the number of private alleles is high. The wide range of genetic distances within group B suggested that most of the splitting events within this group seem to have occurred in a wide time-span, which can be dated back to climatic shifts and marine transgressions during the Pliocene-Pleistocene glaciations. Extinctions and recolonizations during several glaciation episodes in the last Pliocene and during the Quaternary could explain both the observed lack of *A. dentiger* in many potentially colonizable habitats within its area and the varying degrees of genetic differentiation observed in the group B. Since these processes are much older than
the colonization by populations of group A, we would expect the populations from group B to have partly rebuilt their genetic variability. Indeed, we did observe a statistically significantly higher heterozygosity in the populations from group B than from group A (Mann-Whitney Z=2.07; p<0.05 at two-tailed test). Furthermore, surface and cave populations of group B are also differentiated. Within group B, surface populations show a higher level of average heterozygosity than the value observed in the cave ones, which is in turn comparable to the value observed in group A (Tables 3a,b). So, in group B, increasing genetic variability occurred in surface populations only, while cave populations seem to be influenced by the effects of genetic drift or by some form of stabilizing selection. This scenario is consistent with the genetic distance values observed. In fact, group A and B are genetically distinct (Table 4), and in group B average genetic distance among cave populations is much higher than the value observed for surface ones (Tables 3a,b).

The geographical patterns of alleles shared by two and three populations might be interpreted as a trace of an ancestral polymorphism reduced by genetic drift due to extinction dynamics (Gentile and Sbordoni, 1998). In fact, the higher the number of populations sharing the same allele, the more unlikely it is that this allele arose by recurrent mutation in those populations. Populations sharing these alleles are separated by geographic distances up to 250 Km, suggesting that extinction events might have occurred over a wide geographic scale.

Table 3a – Level of population diversity within groups A and B: Average genetic distances (Nei'72) and observed heterozygosity within cave and surface populations.

<table>
<thead>
<tr>
<th></th>
<th>Cave (A)</th>
<th>Surface (A)</th>
<th>Cave (B)</th>
<th>Surface (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D) (Nei'72)</td>
<td>0.141</td>
<td>0.105</td>
<td>0.465</td>
<td>0.186</td>
</tr>
<tr>
<td>(H_0)</td>
<td>0.082</td>
<td>0.089</td>
<td>0.096</td>
<td>0.129</td>
</tr>
</tbody>
</table>

Table 3b – Differences (\(\Delta\)) between levels of population diversity within groups A and B: The upper values is the triangular matrix are \(\Delta D\); the lower ones are \(\Delta H_0\).

<table>
<thead>
<tr>
<th></th>
<th>Cave (A)</th>
<th>Surface (A)</th>
<th>Cave (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface (A)</td>
<td>0.036 ns</td>
<td>0.008 ns</td>
<td></td>
</tr>
<tr>
<td>Cave (B)</td>
<td>0.324 **</td>
<td>0.360 **</td>
<td>0.015 ns</td>
</tr>
<tr>
<td>Surface (B)</td>
<td>0.045 ns</td>
<td>0.081 *</td>
<td>0.279 **</td>
</tr>
<tr>
<td></td>
<td>0.047 **</td>
<td>0.040 **</td>
<td>0.033 *</td>
</tr>
</tbody>
</table>

(*) \(p[D=0]<0.05; (**) p[D=0]<0.01
Table 4 – Level of population differentiation between groups A and B: Average genetic distances (Nei, 1972) between cave and surface populations

<table>
<thead>
<tr>
<th></th>
<th>Cave (A)</th>
<th>Surface (A)</th>
<th>Cave (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface (A)</td>
<td>0.118 ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cave (B)</td>
<td>0.710 **</td>
<td>0.671 **</td>
<td>0.371 *</td>
</tr>
<tr>
<td>Surface (B)</td>
<td>0.624 **</td>
<td>0.633 **</td>
<td></td>
</tr>
</tbody>
</table>

(*) p(D=0) < 0.05; (**) p(D=0) < 0.01

Genetic relationships

The genetic distances between populations morphologically belonging to *A. dentiger* show a wide spectrum of values, including many values higher than 1. Thorpe (1983) suggested that genetic distance values higher than 0.163 between allopatric populations indicate that they belong to different species. If we accept this suggestion, most populations of *A. dentiger* are different species. As already pointed out (Lessios and Weinberg, 1994) there is no theoretical reason to consider the cut-off value indicated by Thorpe as an unambiguous threshold for speciation. The inclusion of species morphologically differentiated as outgroups allows us to calibrate the amount of genetic divergence that can be revealed by allozyme data, providing a “within taxon” standard which is useful to establish a threshold for speciation. Most of the average genetic distances between *Androniscus* species are of the same order of magnitude as many distances between populations of *A. dentiger*.

In contrast with the high degree of genetic divergence, morphological differentiation in *A. dentiger* does not show a degree of geographic variation useful to study the systematic relationships among populations (Vandel, 1960; Gentile, 1994).

In the last twenty years the number of cryptic/sibling species which have been claimed to occur in various taxa is greatly increased. Genetic, ecological and behavioral data are often used and sometimes combined to test the actual differentiation between putative species. In particular, most genetic studies of cave dwelling isopods, both aquatic and terrestrial, revealed the occurrence of high genetic distance values between morphologically indistinguishable populations as reported from studies on *Typhlocirolana* (Caccone et al., 1986), *Stenasesellus* (Messana et al., 1995), *Oritonisculus* (Cobolli Sbordoni et al., 1995) and *Trichoniscus* (Cobolli Sbordoni et al., 1997). Since it has been possible to evidenciate that reproductive isolation may occur in allopatry as a by-product of a high degree of genetic differentiation (Coyne and Orr, 1989), it would appear reasonable that speciation events may occur more frequently than has been though. The high levels of genetic divergence we observed suggest that *A. dentiger* could be probably considered as a complex of cryp-
ANDRONISCUS DENTIGER COMPLEX FROM CENTRAL ITALY

We could identify two genetically differentiated groups of populations (A and B). Additionally, in the group B, most of genetic distances observed between populations are much higher than the values reported for morphologically distinguishable species. However, it remains to be assessed how many species *A. dentiger* complex might include. This appears to be a difficult task, since breeding experiments carried out on other Peracarids showed that the paradigm “high genetic distance - high degree of reproductive isolation” does not hold always (Scheepmaker, 1990).

![NJ Tree](image)  
**Fig. 3** - Genetic relationships between *Androniscus* species. a) Neighbor-join (NJ) tree. The number at the nodes of NJ is the number of times the cluster at the right of the node occurs out of 100 bootstrap repetitions. Only bootstrap values higher than 50% are shown. b) Maximum parsimony tree (MP). The number at the nodes of MP is the number of times (percent) that the cluster at the right of the node occurs out of all most parsimonious trees found. Slashes represent the changes of character states between two contiguous nodes.

Both the NJ tree, and the parsimony analysis are in agreement with the multivariate analysis (FCA). However, neither NJ or MP tree (Fig. 3) is helpful to assess the genetic relationships between the different species of *Androniscus* studied. They suggest that *A. dentiger* is polyphyletic. However, bootstrapping and Templeton’s test do not support the polyphyletic origin of *A. dentiger*, which indeed appears to be unreasonable even from a biogeographical point of view. In fact, among all the *Androniscus* (*Dentigerocen-
GABRIELE GENTILE and GIULIANA ALLEGRUCCI

Fig. 4 – Geographic variation of allele frequencies in *A. dentiger* of Central Italy. The contour map has been obtained by interpolating the values of the coordinates on the first axis after a Factorial Correspondence Analysis. Each tone of color corresponds to an increment equal to 0.2 on the first axis. The dark area identifies the group A, while group B is represented by the white area (Redesigned from Gentile, 1998).

*niscus*) species, only *A. dentiger* occurs in Central Italy, the range of the other congeneric species being strictly limited to the North and North-Eastern Prealps. The difficulty to assess robust genetic relationships between populations and species of *Androniscus* might be explained by the high degree of genetic differentiation found.

Further investigations by using a better addressed genetic marker will probably be necessary to investigate the phylogenetic relationships among the species belonging to this genus.
Acknowledgments

Results discussed in this paper are part of Ph.D. thesis of G. Gentile. We are grateful to R. Argano and A. Caccione for reading and discussing an early version of this paper, allowing us to improve it. We are indebted with V. Sbordoni for his criticism and his support in this research.

References


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<th>ME</th>
<th>DVL</th>
<th>TOM</th>
<th>SUB</th>
<th>MIN</th>
<th>PI</th>
<th>APPENDIX</th>
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</table>
Genetic divergence and evolutionary times: calibrating a protein clock for South-European *Stenasellus* species (Crustacea, Isopoda)

Valerio Ketmaier, Roberto Argano, Marina Cobolli and Elvira De Matthaeis*

**SUMMARY**

We studied genetic divergence in a group of exclusively stygobiont isopods of the family Stenasellidae. In particular, we assessed evolutionary relationships among several populations of *Stenasellus racovitzai* and *Stenasellus virei*. To place this study in a phylogenetic context, we used another species of *Stenasellus*, *S. assorgiai*, as an outgroup. *S. racovitzai* occurs in Corsica, Sardinia and in the fossil islands of the Tuscan Archipelago, while *S. virei* is a polytypic species widely distributed in the central France and Pyrenean area. This vicariant distribution is believed to be the result of the disjunction of the Sardinia-Corsica microplate from the Pyrenean region and its subsequent rotation. Since geological data provide time estimates for these events, we can use the genetic distance data to calibrate a molecular clock for this group of stygobiont isopods. The calibration of the molecular clock reveals a roughly linear relationship \( r = 0.753 \) between the genetic distances and absolute divergence times, with a mean divergence rate \( 19.269 \text{ Myr/D}_{\text{Nei}} \) different from those previously reported in the literature and provides an opportunity to shed some light on the evolutionary scenarios of other *Stenasellus* species.

**INTRODUCTION**

Since Darwin and Wallace, biologists have emphasised the crucial importance of geographical isolation in speciation and in the subsequent adaptation and differentiation of species. The present range of several taxa may be seen as a combination of palaeogeographical events and dispersal phenomena. Some organisms have a poor dispersal capability and thus many barriers would constitute insurmountable obstacles to their dispersal. For example, stygobiont animals are strictly bound to the continental subterranean freshwaters, their potential for active or passive dispersal being limited to phreatic systems. Therefore, it can be hypothesised that their distribution reflects more the palaeogeographical and palaeogeological history of the occupied land masses than the active dispersal processes.

One of the most hotly debated topics in evolutionary studies is the possibility to estimate divergence times from genetic and/or morphological data.

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The major constraint to the use of genetic divergence data for the dating of cladogenetic events is the scarcity of related fossil records and their uncertain interpretation for calibration of a molecular clock. Nei (1975) proposed a linear relationship between divergence times and the degree of genetic differentiation. Several studies (for a review, see Sbordoni et al., 1990) have demonstrated that Nei’s equation can be used for relatively small genetic distances ($D<1$), while for higher $D$ values a calibration is needed. Sarich (1977) observed differences in divergence rates among proteins and recognised groups of fast and slow evolving genes. Other studies have demonstrated that a standardised clock for all genes and species does not exist (Scherer, 1990; Gillespie, 1991). Wilson et al. (1977) developed a relative rate test to investigate the existence of a universal clock. However, there is much evidence to reject the hypothesis of a universal clock across very different lineages; the notion of a local clock with lineage-specific rates seems to be more realistic (Nei, 1987). Testing the existence of a molecular clock is of crucial importance in evolutionary studies, but at least one independently timed event has to be used (Busack, 1986). A molecular clock with known relationships among the taxa under study and with well-dated isolation times may provide an accurate estimate, especially when cladogenetic events can be associated with specific well-dated biogeographical scenarios (Caccone et al., 1994; 1997).

According to Alvarez (1972) and Bellon et al. (1977), Corsica and Sardinia separated as a single land mass from the Iberian Peninsula in the Miocene (about 29-27 Mya). After the disjunction, a rotation brought the two islands to their present positions. The separation of Corsica from Northern Sardinia may have begun about 15 Mya and was certainly completed by 9 Mya. The interaction between the Corsica-Sardinia microplate and the Apennines (then being formed) caused the emergence of the Tuscan Archipelago, including the fossil islands presently incorporated in the mainland (e.g. Monte Massoncello, Monti dell’Uccellina, etc.) (from 9 to 7 Mya) (Lanza, 1984). Several taxa are present on each of these land masses (and only on these) with closely related species. Since the potential for dispersal of many of the species is very low or completely absent (especially for cave organisms), it is reasonable to link the cladogenetic events in these lineages with the palaeogeographical history of the area.

Several genetic studies on phylogenetically distinct species showing this vicariant distribution, such as newts and troglobitic beetles, highlighted these tectonic events as an unique model to test the hypothesis of a clock-like behaviour in the increase of the genetic divergence (Caccone et al., 1994; 1997; Sbordoni et al., 1982; 1985; 1990; 1992).
In previous papers (Messana et al., 1995; Ketmaier et al., 1999b), we assessed the level of genetic differentiation among several populations of the three Italian species of the genus *Stenasellus* (*S. racovitzai*, *S. nuragicus*, *S. assorgiai*), which belongs to the exclusively stygobiont family Stenasellidae. Among the South-European species of *Stenasellus*, *S. racovitzai* shows a close morphological affinity with the Pyrenean species *S. virei* (Magniez, 1974). *S. racovitzai* is presently known from two localities in Tuscany, from the water-bearing strata of the hydrographic systems of the Golo River in Corsica and the Rio di Quirra basin in Southeast Sardinia and from an artificial well close to Porto Torres in Northwest Sardinia (Argano et al., 1998). *S. virei* is a polytypic species widely distributed in the Pyrenean area and central France. This vicariant distribution is a remarkable example of allopatric speciation which can be explained in the context of the previously discussed tectonic events.

The aims of this study were:
1. to determine the levels of genetic divergence within and between *S. racovitzai* and *S. virei*;
2. to test the hypothesis of a clock-like nature of the increase of genetic divergence in this group;
3. to calibrate a molecular clock for the genus *Stenasellus* using the palaeogeographical absolute divergence times.

**MATERIALS AND METHODS**

**Collecting sites and study samples**

Eight populations of *Stenasellus* (identified with a three-letter code) were tested for genetic divergence and polymorphisms. The populations of *S. racovitzai* were a part of those analysed in Ketmaier et al. (1999a):
- DAN: from the type locality (Danese cave, Grosseto, Tuscany);
- UCC: from a small cave (Fontanile dei Cavalleggeri) on the Monti dell’Uccellina slope near the sea (Tuscany);
- QUI: from the water-bearing strata of the Rio di Quirra, Sardinia;
- COR: from the water-bearing strata of the Golo River, Corsica.

Two populations of *S. virei virei* and two populations of *S. virei hussoni* were studied:
- VIR: *S. virei virei*, from the water-bearing strata of the Dordou River, S. Izaires, Albi (France);
- TAR: *S. virei virei*, from the water-bearing strata of the Tarn River, Lescluze, Albi (France);
- HUS: *S. virei hussoni*, from the Estelas cave, Pyrenees (France);
- PEY: *S. virei hussoni*, from the Peyrot cave, Pyrenees (France).

Moreover, for the phylogenetic analyses we used a population of *S. assorgiai* (PIT) from the type locality (Pitzu 'e Crobisi cave, Cagliari, Sardinia) as an outgroup.

The collecting sites are shown in Fig. 1.

Live specimens were transported to the laboratory and frozen at -80°C.

![Collecting sites of the *Stenasellus* populations.](image)

**Electrophoretic procedures and data analysis**

Horizontal electrophoresis on 12% starch gel was performed with crude homogenates in TRIS HCl 0.05 M pH 7.5 from each whole specimen. Eleven enzymes, encoded by 15 structural gene loci, were examined: Acid phosphatase (*Acph*, E.C.No.: 3.1.3.2); Alkaline phosphatase (*Aph*, E.C.No.: 3.1.3.1); Esterase (*Est-1, Est-2, Est-3*, E.C.No.: 3.1.1.1); Isocitrate dehydrogenase (*Idh*, E.C.No.: 1.1.1.42); Lactate dehydrogenase (*Ldh*, E.C.No.: 1.1.1.27); Malate dehydrogenase (*Mdih*, E.C.No.: 1.1.1.37); Nothing dehydrogenase (*No-dh*, E.C.No.: 1.6.99.1); Peptidase (*Pep-1, Pep-2*, E.C.No.: 3.4.11); Phosphoglucomutase (*Pgm*, E.C.No.: 2.7.5.1); Phos-
phoexose isomerase \((\text{Phi}, \text{E.C.No.: 5.3.1.9})\); Tetrazolium oxidase \((\text{To}, \text{E.C.No.: 1.15.1.1})\). The procedures used are those in Messana et al. (1995).

The genetic relationships between populations and species were estimated by genetic distance values \((D)\) calculated according to Nei (1978) on the basis of the allele frequencies at 15 common loci and were represented by a dendrogram plotted with the UPGMA clustering method (Sneath & Sokal, 1973). To test phylogenetic hypotheses, we analysed our data set by two different methods: Maximum Likelihood (ML; programme CONTML in Phylip 3.5, Felsenstein, 1993) and Neighbor-Joining (NJ; programme NEIGHBOR in Phylip 3.5, Felsenstein, 1993). The robustness of the trees was tested by the bootstrap method (programme SEQBOOT in Phylip 3.5, Felsenstein, 1993) with 1000 replications for both phylogenetic methods.

The genetic variability of the populations was estimated by \(H_e\) (expected mean heterozygosity under Hardy-Weinberg equilibrium), \(H_o\) (observed heterozygosity), \(P\) (proportion of polymorphic loci according to the criterion of the second most common allele being at least 1\%) and \(A\) (mean number of alleles per locus).

To assess evolutionary rates, we performed a regression of the genetic distances against the absolute isolation times deduced by palaeomagnetic, stratigraphic and geomorphological data (Alvarez, 1972; 1974; Alvarez et al., 1973; Ambrosetti et al., 1979; Bellon et al., 1977; Bonin et al., 1979; Orsini et al., 1980; Cherchi & Montadert, 1982; Esu & Kotsakis, 1983).

Statistical analyses were performed with the BIOSYS-1 programme (Swofford & Selander, 1981), STATISTICA 4.5 for Windows and the PHYLIP 3.5 package (Felsenstein, 1993).

RESULTS

Fifteen inferred loci were consistently scored; three \((\text{No-dh, Pep-2 and To})\) were monomorphic in all study populations, while the remaining twelve were polymorphic in at least one population. Seven fixed alternative alleles were scored among all study populations. Allele frequencies are available from the first author upon request.

A wide range of \(D\) values was found, from 0.009 \((\text{VIR vs TAR})\) to 3.030 \((\text{QUI vs PIT})\). For \(S. \text{racovitzai}\), the genetic distance between UCC and DAN was 0.175, but the value almost reached 1 when the Tuscan (UCC, DAN) populations were compared with the Sardinian (QUI) and Corsican (COR) ones and when the two island populations were compared (QUI vs COR \(= 0.952\)). The genetic distance data are reported in Table 1, while the genetic relationships among populations are represented in the UPGMA dendrogram of Fig. 2. ML and NJ gave trees with identical to-
polymorphies which are summarised in Fig. 3; both trees were rather robust in terms of bootstrap values.

The genetic variability of the populations (Table 2), expressed as Ho, varied from 0.000 (VIR; PIT) to 0.139 (HUS).

The graphical result of the regression analysis of the genetic distances against the absolute times is shown in Fig. 4. The regression line was Myr = 0.234 + 19.262 D_{Nei} and the correlation coefficient was r = 0.753.

Table 1 - Genetic distance values D (Nei, 1978) between study *Stenassellus* populations.

<table>
<thead>
<tr>
<th>Pop.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - UCC</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - DAN</td>
<td>0.175</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - QUI</td>
<td>1.138</td>
<td>1.128</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - COR</td>
<td>0.954</td>
<td>0.686</td>
<td>0.952</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - HUS</td>
<td>1.075</td>
<td>1.011</td>
<td>0.947</td>
<td>1.136</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - PEY</td>
<td>0.994</td>
<td>0.829</td>
<td>0.817</td>
<td>1.025</td>
<td>0.082</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 - VIR</td>
<td>1.081</td>
<td>1.062</td>
<td>1.445</td>
<td>1.383</td>
<td>0.291</td>
<td>0.281</td>
<td>****</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 - TAR</td>
<td>1.165</td>
<td>1.132</td>
<td>1.438</td>
<td>1.474</td>
<td>0.248</td>
<td>0.271</td>
<td>0.009</td>
<td>****</td>
<td></td>
</tr>
<tr>
<td>9 - PIT</td>
<td>1.689</td>
<td>1.607</td>
<td>3.050</td>
<td>1.584</td>
<td>1.752</td>
<td>1.576</td>
<td>1.322</td>
<td>1.373</td>
<td>****</td>
</tr>
</tbody>
</table>

Table 2 - Variability estimates for the study *Stenassellus* populations (Standard Errors in parentheses).

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean sample size per locus</th>
<th>Mean number of alleles per locus (A)</th>
<th>Proportion of polymorphic loci* (P)</th>
<th>Mean heterozygosity Expected** HDYWBG</th>
<th>Mean heterozygosity (H_{e})</th>
<th>Mean heterozygosity (H_{o})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - UCC</td>
<td>22.7 (0.3)</td>
<td>1.3 (0.1)</td>
<td>33.3</td>
<td>0.042 (0.020)</td>
<td>0.047 (0.024)</td>
<td></td>
</tr>
<tr>
<td>2 - DAN</td>
<td>16.0 (0.0)</td>
<td>1.3 (0.2)</td>
<td>20.0</td>
<td>0.067 (0.054)</td>
<td>0.047 (0.035)</td>
<td></td>
</tr>
<tr>
<td>3 - QUI</td>
<td>24.7 (0.3)</td>
<td>1.4 (0.1)</td>
<td>40.0</td>
<td>0.092 (0.054)</td>
<td>0.119 (0.049)</td>
<td></td>
</tr>
<tr>
<td>4 - COR</td>
<td>5.9 (0.1)</td>
<td>1.2 (0.1)</td>
<td>20.0</td>
<td>0.033 (0.018)</td>
<td>0.049 (0.030)</td>
<td></td>
</tr>
<tr>
<td>5 - HUS</td>
<td>14.3 (0.7)</td>
<td>1.7 (0.2)</td>
<td>53.3</td>
<td>0.139 (0.038)</td>
<td>0.196 (0.054)</td>
<td></td>
</tr>
<tr>
<td>6 - PEY</td>
<td>19.0 (0.5)</td>
<td>1.4 (0.1)</td>
<td>40.0</td>
<td>0.079 (0.031)</td>
<td>0.116 (0.042)</td>
<td></td>
</tr>
<tr>
<td>7 - VIR</td>
<td>14.3 (0.7)</td>
<td>1.0 (0.0)</td>
<td>0.0</td>
<td>0.000 (0.000)</td>
<td>0.000 (0.000)</td>
<td></td>
</tr>
<tr>
<td>8 - TAR</td>
<td>13.4 (0.4)</td>
<td>1.4 (0.1)</td>
<td>40.0</td>
<td>0.033 (0.017)</td>
<td>0.087 (0.038)</td>
<td></td>
</tr>
<tr>
<td>9 - PIT</td>
<td>8.7 (0.2)</td>
<td>1.0 (0.0)</td>
<td>0.0</td>
<td>0.000 (0.000)</td>
<td>0.000 (0.000)</td>
<td></td>
</tr>
</tbody>
</table>

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.
** Unbiased estimate (see Nei, 1978)

**DISCUSSION**

We found a certain degree of divergence between the two subspecies of *S. virei*, which could indicate that a process of differentiation is taking place within the species. However, further study of a larger number of populations is needed to elucidate the systematics of *S. virei*.

Preliminary genetic data for *S. racovitzai* (Messana et al., 1995) clearly showed the existence of two well-differentiated species from Tuscany and
Sardinia. In the present study, we also analysed the Corsican population of *S. racovitzai*. Its mean degree of genetic differentiation from the Tuscan and Sardinian populations strongly supports the hypothesis of three well-differentiated species on the three land masses. The lack of morphological differences among cave species that are well differentiated genetically is a common phenomenon (Sbordoni, 1982; Cobolli Sbordoni et al., 1990). This seems to be related to the uniformity of selective pressures acting in cave environments, which leads to high levels of morphological similarity due to parallelism and/or convergence. In a companion paper (Ketmaier et al., 1999 b) we have emphasized that what we presently call *S. racovitzai* is, in fact, a species complex. This strongly supports the hypothesis that the cladogenetic events within this lineage are related to the palaeogeographical connection between Sardinia and Corsica, as well as between Corsica and the Tuscan Archipelago (including the fossil islands).

The ML and NJ analyses indicate an evolutionary scenario based on a vicariance model. *S. racovitzai* and *S. virei* are sister species, and within the *S. racovitzai* complex, there is a close relationship between the Corsican and Tuscan species. This finding is consistent with the palaeogeography of the study area, since Corsica and Tuscany were in contact even after the connection between Corsica and Sardinia disappeared. Thus we believe that a plausible evolutionary scenario leading to the present distribution of the *Stenasellus* species under study can be based on a vicariance model which assumes that an old, widely distributed species became subdivided during the above-mentioned palaeogeographical events.

![Fig. 2 – UPGMA dendrogram constructed on the basis of the observed genetic distances D (Nei, 1978).](image-url)
Fig. 3 – Majority rule consensus tree obtained by ML and NJ bootstrap analyses. Circled nodes include bootstrap percentages of 1000 replications for ML and NJ (first and second value, respectively). Bootstrap values are shown only for nodes for which the two phylogenetic methods had a bootstrap support of 70% or greater.

Fig. 4 – Genetic distances $D$ (Nei, 1978) versus absolute geological isolation times (Myr). Regression line: \(\text{Myr} = 0.234 + 19.262 \ D_{\text{Nei}}\); correlation coefficient $r = 0.753$.

Interestingly enough, the mean level of genetic differentiation shown by our Stenasellus species of the same order of magnitude of that already found among species of the newts Euproctus, distributed in Corsica, Sardinia and Pyrenees (Sbordoni et al., 1990).

The peculiar ecological features of Stenasellidae and the ancient origin of the family itself (Magniez, 1974; 1981) allow us to overcome the kinds
of problems, e.g. severe bottlenecks, that could bias the calibration of the molecular clock. The idea of a clock-like nature of genetic divergence assumes a constant rate of genomic change through time. However, classic models of population genetics predict a temporary loss of variability and an increase of genetic divergence by random genetic drift in populations experiencing a severe bottleneck. Thus in the presence of bottleneck effects, the divergence rate will be overestimated (Nei, 1987). Organisms bound to phreatic or cave water systems have very low (if any) probability of accidental introduction to, or active migration through, unsuitable ecological areas. Heterozygosity data for our island populations do not indicate any loss of genetic variability by random genetic drift with respect to the continental populations. Hence these kinds of events can be excluded for our *Stenasellus* populations.

Although it has generally been recognised that there is a relationship between genetic divergence and time, the nature of this relationship is not very clear (Foley, 1987; Scherer, 1990). We found a strong correlation ($r = 0.753$) between the genetic distances and the absolute palaeogeographical time estimates, which supports the hypothesis of a linear relationship between geological time and genetic divergence.

The divergence rate calculated in this study is 19.269 Myr/D$_{Nei}$. Thus the rate of 14 Myr/D$_{Nei}$ estimated by Sarich (1977) seems to be too slow for our data and would produce misleading evolutionary scenarios. Caccone et al. (1994) stressed that «rate heterogeneities clearly exist at several genomic levels», electrophoretic data on Pyrenean and Sardinian species belonging to the beetle genus *Speonomus* are a further confirmation (Sbordoni et al., 1992), since genetic distance between them is almost twice the genetic distance between the same geographical comparison in *Stenasellus*. Indeed, various studies (Beerli et al., 1996; Hillis & Moritz, 1990) have demonstrated that the clock-like nature of genetic divergence is viable at the level of the same lineage but not across very different lineages. Sarich (1977) divided the set of commonly detected proteins into two main categories — fast and slow evolving loci — and pointed out that the former accumulate electrophoretically detectable substitutions at a rate ten times greater than the latter. In contrast, Thorpe (1989) suggested that there is no clear-cut bimodal distribution of the rates of protein evolution; instead, the differential rates occurring in a data set are probably the result of random sampling from a continuum rather than from well-separated classes. Beerli et al. (1996) emphasised, however, that these differences in the rate of genetic evolution do not falsify the concept of a molecular clock.

We divided our set of scored loci into slow and fast evolving loci according to the procedure proposed by Sarich (1977): dehydrogenase en-
zymes were considered as slow evolving loci, while the other enzymes were
treated as fast evolving loci. This empirical assignment to the two groups
was evaluated by recalibrating the molecular clock without the fast evolving
loci. The mean divergence rate drops from 19.269 Myr/\text{D}_{\text{Nei}} \text{ to } 12.561
Myr/\text{D}_{\text{Nei}}, confirming the existence of rate heterogeneities even at the level
of enzymatic proteins. Several studies (Hillis & Moritz, 1990; Scherer, 1990;
Gillespie, 1991; Wilson et al., 1987) have shown that multiple sub-
stitutions in rapidly evolving parts of the genome (including regions for enzymatic
proteins) can cause significant problems for large divergence times. How-
ever, these authors referred to a time scale longer than 100 Myr. Since our
averaged protein clock spans a maximum of 30 Myr, it is reasonable to as-
sume that it is not affected by these phenomena.

A particularly intriguing aspect of our study is the possibility to de-
velop a mean divergence rate for the genus \textit{Stenasellus} which can then be
applied to populations and species for which no clear palaeogeographical
scenarios are available. The above mentioned problems of rate heterogenei-
ties among different lineages compel one to be cautious in extrapolating a
divergence rate from one group to another. However, as shown by Beerli et
al. (1996), extrapolations within the same genus are permitted.

Despite the degree of error implicit in the method, our calibration of a
molecular clock for the genus \textit{Stenasellus} will be used in the near future to
place in a temporal frame the complex evolutionary scenarios (Ketmaier et
al., 1999 a) presented by some endemic Sardinian species of this genus (i.e.
\textit{S. nuragicicus}, \textit{S. assorgiae}).

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project.

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Long term stability of a terrestrial cave community

Claudio Di Russo *, Gianmaria Carchini *, Mauro Rampini **, Marco Lucarelli * and Valerio Sbordoni *

SUMMARY

We report data on the spatial structure and seasonal variation of the community of Valmarino cave, a medium sized sandstone cave, located a few kilometres from the coast line, in Central Italy. Due to both its habitat features and its relatively recent geological history, Valmarino cave is only inhabited by terrestrial, troglophilic elements, i.e., facultative cave dwellers.

By means of monthly censuses and density plot estimates we have investigated species abundance, diversity and their spatial organization, by considering separately samples from different cave sectors. Homogeneous sampling design allowed to compare series of samplings performed in 1974 and 1994.

On the whole 21 arthropods and one snail species constitute the cave community. Ordination plots resulting from correspondence analyses of monthly samples outline a distinct spatial and temporal structure. Two main sub-communities can be identified: an inner sub-community, mainly represented by eu-troglophilic species, showing a remarkable stability throughout the year and an outer sub-community, mainly represented by sub-troglophilic species, showing strong seasonal variation. Both spatial and temporal vectors show similar importance in shaping the community structure.

An interesting result of this study is the long term stability of both spatial and seasonal components of the community structure which remained almost identical after 20 years, as shown by the comparison of ordination plots obtained from 1974 and 1994 sampling series. Therefore this study provides empirical evidence of a frequently hypothesised, albeit never demonstrated feature of the cave ecosystem.

INTRODUCTION

Caves are generally considered as simplified habitat for the absence of primary production and consequent reduction of the other trophic levels (Poulson & White, 1969). The living organisms they harbour mainly depend on organic matters carried into caves by water, gravity and animals (Barr, 1967).

Caves are also regarded as habitats of great stability owing to their relatively constant micro-climate. Ecological theory predicts that stability

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of the habitat permits community stability (May, 1973). Furthermore stability as other features of communities need to be tested over long time period and must be calibrated on the basis of relative biogeographical and historical scenario of the studied system (Southwood, 1996). In fact, stability of the cave habitat is thought to represent a major factor affecting evolutionary responses in cave dwelling organisms (Vandel, 1964; Sbordoni, 1980; Culver, 1982). These and other features have often awarded caves as «natural laboratories» for the study of evolutionary and ecological processes. However, despite of these favourable conditions, only few and scattered researches were aimed to investigate variation of cave communities in space and time (Turquin et al., 1975; Howarth, 1981; Martin & Oromi, 1986; Poulson, 1992).

Two recent studies showed the occurrence of significant variation in troglobite and cricket-guano communities of Australia and North America, respectively (Humphreys, 1991; Poulson et al., 1995), accounting for a strong dependence of these communities on unpredictable climate changes. These studies raise the question on the generality of the idea of stability of cave communities.

In order to contribute to this important debate, we report a series of data on a long-term investigation of a troglophilic community in the Grotta di Valmarino, a small cave near the Tyrrhenian coast line, in Central Italy. Researches started in 1974 and preliminary results were showed in several scientific meetings (Carchini et al., 1982; 1983; 1992). To test the occurrence of stability in space and time of this cave community, a second series of comparable surveys was carried out in 1994-95.

MATERIAL AND METHODS

Valmarino cave (register number 251 La) is a medium sized sandstone cave located about 150 Km south of Rome, near to the coast line. We have chosen this cave for two important reasons: abundance of fauna and easily detectable cave walls for monitoring.

Detailed data on topography and geology of the cave are reported in Carchini et al. (1982). On the basis of stratigraphic and marine transgression data (Bigazzi et al., 1973) the cave was submerged until 230.000-200.000 years ago and therefore it was not available to colonisation by terrestrial organisms before that time.

Monthly censuses of parietal community (i.e.: the assemblage of organisms currently found on the walls) were carried out using density plot method (Seber, 1973). For this purpose the cave was divided into 6 sectors adapted to the cave topography. One additional sector located outside the
cave entrance was also considered (Fig. 1). The whole area of the sampling surface, as estimated from the cave topographic survey, was 341 square metres. Cave climate was periodically checked. For each sector temperature and relative humidity were recorded using a thermo-hygrometer (Hanna Instruments 8564; °C ± 0.4°C; RH ± 2%). The main cave trophic resource is bat guano that occurs regularly only in the inner sector G on a surface of at least 10 square metres. In order to check variation of this trophic supply, bat colonies and guano surface were monitored during samplings in 1994. Other available resources were dung of other mammals like *Vulpes vulpes*, *Hystrix cretata* and *Rattus norvegicus*.

Analysis of community structure was carried out using multivariate approaches. Species density matrices for each sector and periodical censuses were processed by Factorial Corresponding Analysis (FCA). Abundance comparisons were tested by t-test for dependent samples and Kolgomorov-Smirnov test. Relationships among species as prey/predator and seasonal associations were detected by simple regression analysis. Diversity of periodical samples was estimated by means of the Gini-Simpson index (Gini, 1912).

Fig. 1 – Map of Valmarino cave with the different sampling sectors and range of temperature and humidity recorded.
RESULTS

Cave climate

Temperature and relative humidity were recorded for the two survey periods. As found in most of temperate caves the inner sectors (G and F) appear to be more stable than the other ones, showing an average temperature of 16.5°C and an annual variation limited to 1°C. On the contrary, the sector closest to the entrance (B) showed a thermal variation of at least 5°C, ranging between 14°C and 19°C. As expected, inner and outer sectors reduce their thermal gap in autumn and spring. Finally, no significant difference was observed on the annual trend in temperature between the two sampling periods, 1974 and 1994. Relative humidity in 5 cave sectors was always maintained above 90%. Only the sector B showed lower values (60%).

Community structure

On the whole 22 troglophilic species, including Gastropods and Arthropods, appear to constitute the framework of the parietal community of Valmarino cave. We have not considered the species occasionally recorded in the cave. In Table 1 the list of species sampled is reported. We used the terms eutroglophile and subtroglophile, following a terminology currently used by European biospeleologists (Ruffo, 1959), where the first ones are considered more strictly linked to cave for reproduction, not necessarily showing troglomorphic features (i.e. blindness, depigmentation and elongation of appendages). On the contrary the subtroglophiles may occur periodically in cave without any particular morphological adaptation to cave life. Each species has also been characterised by its trophic role (Root, 1967). We have not found any strictly troglobite species and the ratio Eutroglophile/Total species was 0.27. Concerning the trophic structure we found an high percentage of Predators (53%) among species feeding inside the cave. The most abundant species were, in decreasing order, *Culex pipiens*, *Limonia nubeculosa*, *Porcellionides pruinosis* and *Dolichopoda geniculata*.

Fig. 2, represents the ordination plot from a FC analysis performed on the relative abundance of any species in each period and sector. Species show a distinct structure and different levels of clustering, largely corresponding to their dependence on season and sector, indicating their degree of troglophily. It is easy to argue that the first axis mainly represent seasonal variation, while the second axis represents spatial location, i.e.: sector. Eutroglophilic species as *Oxychilus draparnaudi*, *Nesticus eremita*, *Dolichopoda geniculata*, the endemic *Tegenaria marinae* and *Callipus foe-
tidissimus sorrentinus are closely grouped, toughly corresponding to the inner sectors G and F. Location of this cluster near the centre of the first axis accounts for a modest, almost irrelevant, seasonal variation. On the contrary subtroglophilic species are scattered across sectors progressively close to the entrance. Among these, moths (A. spectrum and H. obsitalis) and mosquitoes (C. pipiens), which tend to hibernate into the cave, show on the first axis an opposite location to Limonia nubeculosa, which behaves as an aestivating species. Only few species were recorded on the walls of the external sector (O), namely the common spiders Tegenaria parietina and Pholcus phalangioides.

Table 1 – List of species sampled in Valmarino cave

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Symbols</th>
<th>Category</th>
<th>Trophic role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastropoda</td>
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<td></td>
<td></td>
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<tr>
<td>Oxychilus draparnaudi</td>
<td>OXY</td>
<td>Eutroglophile</td>
<td>Predator</td>
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<tr>
<td>Chilopoda</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Scutigera coeleoptrata</td>
<td>SCU</td>
<td>Subtroglophile</td>
<td>Predator</td>
</tr>
<tr>
<td>Diplopoda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callipus f. sorrentinus</td>
<td>CAL</td>
<td>Eutroglophile</td>
<td>Detritivore</td>
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<tr>
<td>Aranae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pholcus phalangioides</td>
<td>PHO</td>
<td>Subtroglophile</td>
<td>Predator</td>
</tr>
<tr>
<td>Tegenaria parietina</td>
<td>PAR</td>
<td>Subtroglophile</td>
<td>Predator</td>
</tr>
<tr>
<td>Tegenaria marinae</td>
<td>MAR</td>
<td>Eutroglophile</td>
<td>Predator</td>
</tr>
<tr>
<td>Meta merianae</td>
<td>MER</td>
<td>Subtroglophile</td>
<td>Predator</td>
</tr>
<tr>
<td>Nesticus eremita</td>
<td>NES</td>
<td>Eutroglophile</td>
<td>Predator</td>
</tr>
<tr>
<td>Isopoda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcellio dilatatus</td>
<td>EUP</td>
<td>Eutroglophile</td>
<td>Detritivore</td>
</tr>
<tr>
<td>Armadillidium vulgare</td>
<td>ARM</td>
<td>Subtroglophile</td>
<td>Detritivore</td>
</tr>
<tr>
<td>Porcellionides pruiniosus</td>
<td>MET</td>
<td>Subtroglophile</td>
<td>Detritivore</td>
</tr>
<tr>
<td>Orthoptera</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gryllomorpha dalmatina</td>
<td>GRY</td>
<td>Subtroglophile</td>
<td>Scavenger</td>
</tr>
<tr>
<td>Dolicichopoda geniculata</td>
<td>DOL</td>
<td>Eutrolphile</td>
<td>Scavenger</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Apopestes spectrum</td>
<td>APO</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Monopis sp.</td>
<td>MON</td>
<td>Subtroglophile</td>
<td></td>
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<tr>
<td>Hypena obsitalis.</td>
<td>HYP</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Microlepidoptera</td>
<td>MIC</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Diptera</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Culex pipiens</td>
<td>CUL</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Limonia nubeculosa</td>
<td>LIM</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Brachyccera</td>
<td>BRA</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Nematocera</td>
<td>NEM</td>
<td>Subtroglophile</td>
<td></td>
</tr>
<tr>
<td>Psychodidae</td>
<td>PSY</td>
<td>Subtroglophile</td>
<td></td>
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</tbody>
</table>

Relationships between species were tested by simple regression analysis on the whole sample (1974-1994). Statistically significant correlations were found between abundances of species pairs, mostly due to the occurrence of similar seasonal behaviour. In two cases, however, such correlation might be
interpreted as the effect of a prey/predator relationships as in the pair *Pholcus-Porcellionides* \((r=0.67\) for 1974 and \(r=0.71\) for 1994, \(p<0.01\)) and in the pair *Culex-Scutigera* \((r=0.86\) in 1974, \(p<0.001\), and \(r=0.51\) in 1994, \(p<0.05\); see Fig. 3). Interesting enough is the seasonal vicariance between *C. pipiens* and the other most common dipteran species *Limonia nubeculosa*, indicating the occurrence of a marked temporal displacement of cave roosting of the two species (Fig. 3). In fact, the mosquito *C. pipiens* inhabits the cave for winter dormancy of pregnant females, while *L. nubeculosa* enter the cave only in late spring for aestivation.

**Community structure variation**

Variation of the community structure in time and space is synthetically represented in the ordination plots reported in Fig. 4. These two plots, obtained from Correspondence Analyses of monthly censuses from different sectors, show a remarkable similarity between the two surveys of 1974 and 1994. In both surveys, samples are roughly arranged on a triangle, where the superior vertex corresponds to the inner sectors (G and F) and the basis corresponds to the outer sectors (mainly A, B and C). In fact, the first axis mainly traces seasonal variation of community samples, while the second axis traces their spatial arrangement. In both surveys samples from inner
sectors are closely grouped, showing a very small degree of seasonal variation. On the opposite samples from the outer sectors are largely spread on the first axis, showing substantial high seasonal variation in both surveys. As expected, samples from the intermediate sector E, occupy intermediate locations in respect of both axes, showing a lesser extent of seasonal variation than outer samples.

Variations of the Gini-Simpson diversity index are reported and compared in Fig. 5. Similar trends in seasonal variation are evident in both surveys, where most of the monthly values average 0.8, but two major negative peaks occur in summer and late fall, corresponding to the massive arrival of *L. nubeculosa* and *C. pipiens*, respectively.

Finally, species abundance was also compared (Fig. 6). On the whole a general decrease for most taxa was observed, even if no local extinction apparently occurred. Such decline significantly affected eutroglophilic species and predators, as indicated by the t-test analysis for dependent samples (see asterisks on the histograms of Fig. 6). Decrease of bat guano was also estimated to occur by at least 50%. It was apparently due to a consistent reduction of the bat colony, mainly represented by *Rhinolophus euryale* and *Miniopterus schreibersi*, which dropped roughly by a factor 10, from an average of 300-400 individuals in 1974 to 35-40 in 1994.

Fig. 3 – Variation in monthly abundance of *Culex pipiens* (PIP), *Limonia nubeculosa* (LIM) and *Scutigera coleoptrata* (SCU): a comparison between 1974 and 1994.
However, frequency distributions of species throughout the whole cave, did not show statistically significant variation between the two surveys, as supported by means of a Kolgomorov-Smirnov test (p>0.1), indicating that the relative composition of community remained similar and stable in a time span of 20 years.

DISCUSSION

Periodical samples investigated by means of density plot method allowed us to describe the structure of the parietal community at Valmarino cave and its variation in space and time. On the whole the species composi-
tion of this community appears quite similar to other communities dominated by troglophilic species, inhabiting caves and man made hypogeans at a similar elevation in Central Italy (Capolongo, 1969; Sbordoni, 1971). The absence of troglobitic species could be explained on the basis of two main factors. The first is related to the sandstone origin of the cave and the lack of crevices and fissures preventing migration of troglobites from other cave systems and/or the underground superficial compartment (M.S.S. as defined by Juberthie et al., 1980). The second factor concerns evolutionary time and palaeogeographic evidence. In fact, stratigraphic data testify that Valmarino cave was submerged until 230,000-200,000 years ago (Bigazzi et al., 1973). However, even more recent marine transgressions occurred in the late Pleistocene could have left little time and chance for troglobitic evolution. Accordingly, the cave community shows a rather low ratio eutroglophiles/total species (0.27), indicating its low degree of specialisation.

Fig. 5 – Monthly variation of the Gini-Simpson diversity index calculated for the cave community at Valmarino cave.
The high percentage of predators found in the cave suggests that the community structure is basically controlled by predation. The role of predation in shaping the seasonal pattern of Valmarino cave community is also revealed by the occurrence of statistically significant correlations, ascertained in both sampling periods, between pairs of interacting taxa, such as *Scutigera coleoptrata* vs. *Culex pipiens*, and *Pholcus phalangioides* vs. *Porcellionides pruinosis*. Similar patterns were found in a cave of North West Cape Peninsula, W. Australia (Humphreys, 1991) and in the Mammoth cave, in Kentucky (Kane, 1974). In both cases the terrestrial communities appeared to hold many predator species. Evidence of narrow prey-predator relationships was also found between the carabid beetle *Neaphenops tellkampfi* and the cricket eggs of *Hadenoecus subterraneus* (Kane, et al., 1975).

Several species exhibit a strong spatial structure and show stable associations in certain sectors, depending upon their degree of tolerance and the stability of microclimate. As expected, the most specialised cave organisms like *Dolichopoda geniculata*, *Oxychilus draparnaudi*, *Callipus f. sorrentinus*, *Nesticus eremita*, and others are toughly associated to the inner sectors G and F. For some of these species reproduction and/or ovideposition only occur in these sectors whose climatic conditions appear to meet optimal requirements for some species. In the case of the cave cricket *D. geniculata*, the average temperature of the sector G (16 °C) is very close to the thermal...
optimum of the embryonic development, as established by laboratory experiments (Di Russo & Juberthie, 1995). On the other hand, other species in the community show a distinct temporal pattern of seasonality in their occurrence in the cave. Species relationships can be either synchronous, as shown by the co-occurrence in winter of *Culex pipiens* and *Scutigera coleoptrata*, or allochronic, as in the two most common Dipterans: *Culex pipiens* and *Limonia nubeculosa*, which show seasonal vicariance in cave occupancy, due to their different timing of adult dormancy.

Such occurrence of strong spatial and temporal structures in the community of Valmarino cave is clearly reflected in the ordinations resulting from correspondence analyses which outline the existence of two opposite components within the cave community, i.e.: a component of subtroglophilic organisms characterised by strong seasonal behaviour mostly represented in the outer sectors, and a component of eutroglophilic species almost confined to the inner sectors of the cave. Interestingly enough, both the spatial and the time vectors show similar importance in determining the structure of this community.

Given these two major determinants of the community structure, the most important result obtained in this study is the high level of stability, over 20 years, showed by this cave community. The comparison of ordination plots of species samples, in space and time, shows surprisingly similar patterns in both periods of sampling (1974 and 1994).

The same conclusion can be drawn by comparing the seasonal behaviour of the Gini-Simpson diversity index, also accounting for a long term stability of the Valmarino cave community in both species composition and relative numbers of individuals in the various sectors of the cave.

Long-term stability of cave communities is a feature claimed since early studies in biospeleology (Racovitza, 1907; Jeannel, 1926; Vandel, 1964), and this view inspired the refugia theory of cave evolution as well as concepts and ideas largely shared among early biospeleologists who considered troglobites as relics or even living fossils (Jeannel, 1943). On the other hand, as recently found by other authors (Humphreys, 1991; Poulson et al., 1995), environmental unpredictable stress could strongly affect stability of cave communities. In these examples, variation in resource input by cave crickets and water, respectively, determined significant qualitative and quantitative changes in the cave community.

Interestingly enough, an important reduction in energy supply also occurred in Valmarino cave, where the main resource, represented by bat guano deposits, met a substantial decrease. However, apparently, this change did not affect the qualitative composition of the community structure. Actually most
of the eutroglophilic and predator species showed a sensible decrease in number, however no instance of local extinction was detected over twenty years. This findings suggest that the troglophilic community of Valmarino cave is subjected to a certain degree of «homeostasis» that allows to modulate its quantitative composition without altering its fundamental structure.

The question arises on how general is the pattern revealed in this study. Are caves really stable and predictable habitats in the long term? To what extent this is a feature of the cave environment? Unfortunately long term studies of community structures are still scanty. Lawton & Gaston (1989) recorded the abundance of phytophagous insects on two patches of bracken over seven and fifteen years and found that their relative abundance changed hardly at all. Furthermore experimental studies of disturbance on some stream invertebrate community showed that quality and quantity of species declined with increasing disturbance intensity (Lake et al., 1989; Death, 1996). It could be expected that any disturbance applied on these environmental fragments result in community changes followed by more or less quick recovering, due to recolonization and migration between patches. Caves are more isolated habitats and are subjected to comparatively stable climatic regimes. In particular, limestone caves are thought to be almost free from medium frequency disturbance (Margalef, 1993) and our micro-climate data from various sectors of the Valmarino cave did not show any significant change over 20 years. Therefore results from this study seem to support May's view (May, 1973) that community stability depends chiefly on the stability of all factors that contribute to their physical environment.

ACKNOWLEDGEMENTS

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REFERENCES


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