Patterns of genetic diversity within selected subterranean fauna of the Cape Range peninsula, Western Australia: systematic and biogeographic implications

M. Adams[1] and W.F. Humphreys[2]

[1] Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, South Australia, 5000, Australia.

[2] Department of Terrestrial Invertebrates, Western Australian Museum, Francis Street, Perth, Western Australia 6000, Australia.

Abstract

Detailed and informative systematic frameworks for the species under consideration are an essential prerequisite for any comprehensive biogeographical analysis of the Cape Range peninsula. The molecular technique of allozyme electrophoresis has been used to provide such a framework for six separate groups of subterranean fauna occupying the peninsula. The allozyme data are used to assess patterns of genetic diversity within and amongst a troglophilic philosciid isopod, *Laevophiloscia yalgoonensis*, and five troglobitic groups, the schizomid *Draculoides vinei*, the paradoxosomatid millipedes *Stygiochiropus* spp., an undescribed genus of melitid amphipod, *Stygiocaris* spp. shrimps, and the blind gudgeon *Milyeringa veritas*. The implications of the data for species delineation, the reconstruction of evolutionary relationships, the estimation of times of divergence, and various aspects of intraspecific diversity are discussed in some detail.

The major outcomes of the study are :- (1) confirmation of the existence of multiple species within the millipedes, amphipods, and shrimps; (2) an indication that additional discrete lineages occur within single morphospecies of millipedes and amphipods; (3) the postulation of a designated sequence of cladogenic events within each troglobitic group; (4) the recognition of three, geographically-based, genetic provinces within both the millipedes and amphipods, but not within the schizomids; (5) the existence of substantial population substructuring within most troglobitic species; (6) the suggestion of "eastern" and "western" components to the fauna occupying the coastal plain, based on the patterns of diversity displayed by the shrimps and the gudgeons; and (7) the suggestion that gene flow is restricted or absent in most of the troglobites. These conclusions all have some relevance to other biogeographic studies outlined in this volume.

Introduction

The purpose of this symposium is to bring together scientific information available on the Cape Range peninsula and to interpret this information, wherever possible, from a biogeographic perspective. This of course will be done in a variety of ways in this symposium, using a number of quite different sets of biological, geological and hydrological data. However, one feature that is common to all biogeographical interpretations of biological data is the importance of a sound systematic framework underpinning the organisms under discussion. Major uncertainties or inaccuracies in the systematic framework being used will severely confound the formulation of real biogeographic perspectives. Thus a key first step in biogeography is the availability of an accurate and informative systematic structure for the species under consideration, a factor often ignored in biogeographical texts (Myers and Giller 1988).

One major advance in biology over the past 20 years has been the incorporation of a variety of molecular technologies as a significant component of systematics. Molecular systematics has the potential to provide important additional information in situations where morphological analysis is uninformative or ambiguous. This paper outlines the contribution that molecular techniques have made to our understanding of the local biogeography of Cape Range peninsula. The technique of allozyme electrophoresis has been used to examine patterns of genetic diversity within and amongst selected elements of the subterranean fauna. This information can be used to provide an independent framework for the reconstruction of the recent biogeographic history of these faunal groups.

The fauna selected for study

As outlined elsewhere (Humphreys 1993), the subterranean fauna play a significant role in any biogeographic assessment of the Cape Range peninsula. The initial thrusts of the allozyme work were to examine population differentiation and gene flow within a number of the more common morphospecies of troglobite found on the peninsula, whilst at the same time assessing whether any cryptic species were present amongst these groups. As work progressed, other components of the systematic hierarchy within particular groups were addressed on an *ad hoc* basis. Some of these data have already been published (Humphreys *et al.* 1989; Humphreys and Adams 1991; Humphreys and Shear 1993), and others are in preparation. In this paper we provide an overview of these studies, omitting the detailed presentation that would ordinarily characterize an allozyme study.

Of the subterranean fauna resident on the Cape Range peninsula, a number of groups were identified as suitable for allozyme characterization. In general, taxa were chosen to satisfy as far as practicable the following criteria:- (1) those which survey work revealed to be abundant enough to permit independent molecular and morphological characterizations consistent with their conservation, (2) those of sufficient physical size to permit allozyme analysis, and (3) to maximize the phylogenetic, geographic, and ecological diversity of taxa examined.

Species from six animal groups were targeted for analysis. The groups were:- non-troglobitic philosciid isopods, and troglobitic paradoxosomatid millipedes, schizomids, atyiid shrimps, melitid amphipods, and blind gudgeons. Table 1 gives specific taxonomic and ecological details for the morphospecies examined from within each group. The selection of taxa allowed various classes of comparison, for example troglobitic versus non-troglobitic, invertebrates

Table 1. A taxonomic and ecological overview of the six faunal groups used in this study. A summary of the allozyme data available for each group is also presented.

Taxonomy			Ecology				Allozymes	
Group	Genus	Morphospecies	Troglobite	Aquatic	Cape Range	Specimens	Sites	Loci
Isopods	*Laevophiloscia*	*L. yalgoonensis* Wahrberg	-	-	+	23	5	32
Schizomids	*Draculoides*	*D. vinei* (Harvey)	+	-	+	80	18	23
Millipedes	*Stygiochiropus*	*S. communis* (Humphreys & Shear)	+	-	+	271	36	24
		S. sympatricus (Humphreys & Shear)	+	-	+	7	1	18
Amphipods	undescribed	sp1 - Cape Range	+	+	+	28	4	34
		sp2 - coastal plain	+	+	-	5	1	21
Shrimps	*Stygiocaris*	*S. lancifera* Holthuis	+	+	-	21	4	30
		S. stylifera Holthuis	+	+	-	25	4	30
Gudgeons	*Milyeringa*	*M. veritas* Mees	+	+	-	29	7	43

Table 2. The sample sizes used and caves or wells sampled for all faunal groups. As for Figure 1, sites are listed numerically, omitting the "C-" prefix. Two additional localities were used; Barrow Island (shrimps, N=5) and well C-413 on the coastal plain (amphipods sp2, N=5).

Group	15	18	24	25	27	47	56	62	64	65	68	79	102	103	106	107	111	118	119	126	149	151	154
Isopods		8													3	4				4			
Schizomids	2	8											2	4	8	2		6		12			7
Millipedes	10	14				9	5	1	3	9	4	11		16	10	8	9	1	1	12		2	7
Amphipods		4							9					4									
Shrimps			7	5	16																4		
Gudgeons				5	3																5		

Group	156	159	161	162	163	167	171	207	215	227	250	254	256	260	261	263	273	274	278	281	312	361	362
Isopods						4																	
Schizomids	4	7		4		4					3	6	3			3			1				
Millipedes	9	1	9	7	10	6	9	10	10	13		9	10	6	1	11			11	4	10		
Amphipods					9																		
Shrimps																	6					3	
Gudgeons																	6	4				3	2

versus vertebrate, terrestrial versus aquatic, and a coastal plain versus Cape Range distribution. We hereinafter refer to these groups mostly according to the common names listed in Table 1, except where a distinction needs to be made within a group.

The caves or wells represented in this study are indicated in Table 2, together with the numbers of animals sampled for each group. The relative locations of these caves or wells are presented in Figure 1. There are two major geographic regions, a small number of wells and caves along the coastal plain which contain the shrimps, gudgeons, and one of the species of amphipod, and numerous caves within Cape Range that are inhabited by the isopods, schizomids, millipedes and the other amphipod species (in the four caves containing standing water). The caves are spread over an approximately 40 km by 10 km area of Cape Range, with most found concentrated within a middle area of about 22 km by 8 km. This middle area is traversed by a number of deep gorges which cut through the cavernous limestone into the underlying non-cavernous strata (Humphreys 1993). For convenience, we consider these gorges to delineate the area into three discrete geographic provinces, hereafter referred to as the northern, central and southern provinces (Figure 1).

As can be seen from Table 2, the 76 populations are represented mostly by small sample sizes (mean 6.2, range 1-16). Such sample sizes may be adequate for delineating species boundaries, determining evolutionary relationships, and assessing divergence times, but will not allow a comprehensive assessment of intraspecific differentiation (Richardson *et al.* 1986). Nevertheless, these sample sizes do allow any major intraspecific trends in genetic differentiation to become readily apparent.

Specimens were snap frozen in liquid nitrogen in the field and transported to the electrophoresis laboratory on dry ice. Samples were collected opportunistically over several years and electrophoresed in batches as they became available. Where necessary, the results of two or more separate runs on the same group were integrated by including samples recollected from key populations as part of any subsequent batch. The general electrophoretic procedures and laboratory techniques used to generate the allozyme data are detailed in Richardson *et al.* (1986).

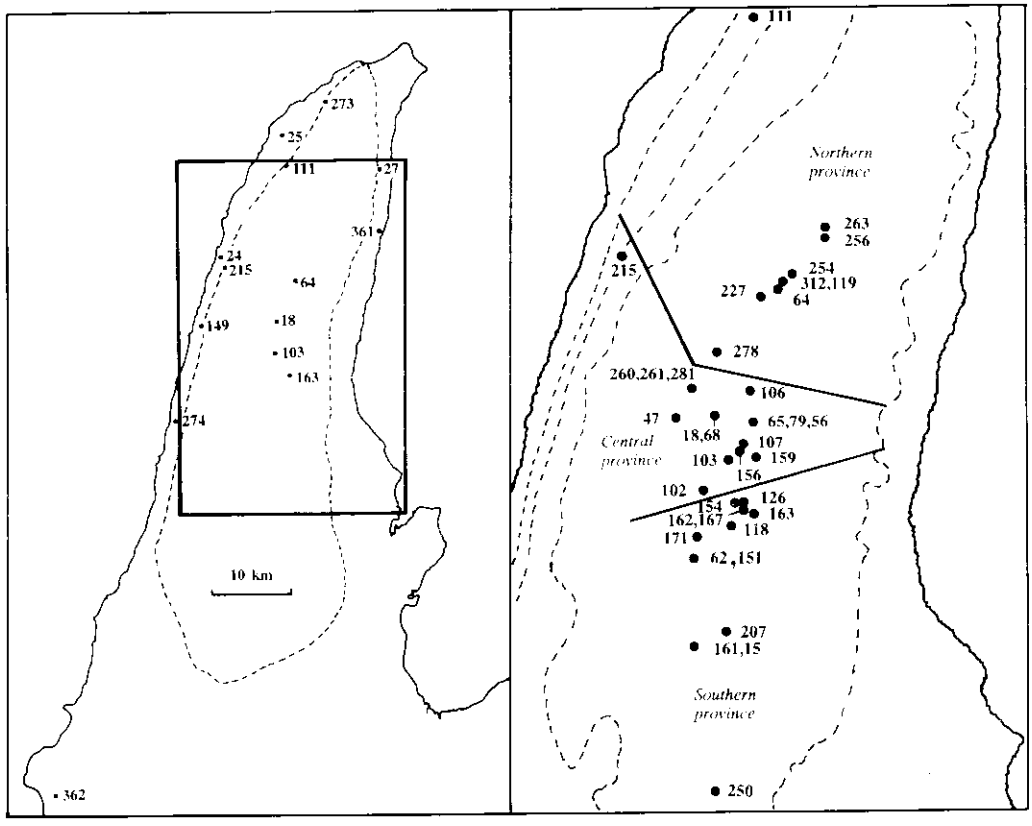

Figure 1. a) Map of Cape Range peninsula showing the location of caves and wells used in the study (well C-413 not shown). Figure 1b) - an expanded section of the inset of Cape Range, showing the geographic arrangement of all caves. Lines are used to specify the boundaries of the northern, central and southern geographic provinces (see text). The broken lines delineate a) the location of Cape Range and b) the surface exposure of Tulki Limestone.

Molecules and morphology in systematics

Before proceeding to a discussion of genetic diversity within the fauna of Cape Range peninsula, it is instructive to first present some background perspectives in systematics. This background will relate directly to the parameters of genetic diversity discussed for individual groups, and will assist in the correct interpretation of any patterns of diversity that unfold. A central theme of this discussion will be the contribution that molecular data can make to the resolution of ambiguities in particular parts of any systematic framework already derived from morphological data.

There are four overlapping but identifiably different areas of systematics which impinge directly on biogeography. These are :- (1) the recognition and delineation of species within a group, (2) the hierarchical determination of evolutionary relationships within and between groups, (3) the estimation of times of divergence for taxa, and (4) the documentation and analysis of intraspecific diversity. Historically, these four areas have all been assessed using morphological analysis, often in combination with ecological, behavioural, or fossil data.

However, it is becoming increasingly recognised that morphological assessment alone cannot provide all of the answers required in these areas (Baverstock 1990).

Modern approaches to systematics now feature the integration of molecular data with more traditional methods of study. As might be expected, the juxtaposition of two contrasting methodologies within the one discipline has lead to a vigorous discussion in the literature about the relative merits of the two approaches (Moritz and Hillis 1990). Unfortunately much of this discussion has focused on trying to determine which set of characters is "better" than the other (e.g. Patterson 1987; Kluge 1983; Sibley and Ahlquist 1987), rather than looking at how best to integrate the two.

The most important concept to grasp when comparing morphological and molecular evolution is that each may take place quite independently of the other (Baverstock and Adams 1984; Hillis and Moritz 1990). The two processes are generally subject to very different evolutionary pressures and governed by differing sets of rules (Wilson *et al.* 1974, 1977). Morphological and molecular data sets can both contribute greatly to the ultimate determination of systematic frameworks (Hillis 1987). Neither is innately superior to the other, although one may be more suitable than the other when addressing any given systematic problem (Hillis and Moritz 1990). As a general principle, studies which include both morphological and molecular approaches are likely to provide much more powerful and robust descriptions of biological diversity than those using only one approach. We have followed this rationale in our efforts to characterize the subterranean fauna on Cape Range peninsula.

A variety of molecular techniques is now available for incorporation into any systematic study. There are three broad types of technology (Hillis and Moritz 1990); those utilizing chromosome analysis, those addressing genetically-determined variation in proteins (allozyme electrophoresis and immunological techniques), and those which directly measure genetic variation in the nucleic acids (DNA hybridization, DNA and RNA sequencing, and restriction enzyme analysis). Of these technologies, allozyme analysis is recognised as being the most cost-effective and thorough way of simultaneously addressing a range of systematic issues in recently-evolved species and species groups (Richardson *et al.* 1986; Moritz and Hillis 1990). As such we will be focusing on allozyme studies as the molecular reference point in this paper.

Delineation of species

When considering the delineation of species boundaries, it is important to determine first what particular species concept is to be used. Species concepts can be broadly categorized into three major schools of opinion, namely "evolutionary", "biological", and "phylogenetic" (Mayr 1970; Otte and Endler 1989; Frost and Hillis 1990). Traditionally, systematists have by necessity focused mainly on the use of an evolutionary species concept, using the only characters that were available (e.g. morphological characters supplemented by the occasional ecological, behavioural or physiological information). As a consequence, many species have been described primarily by recognizing discontinuities in the grades of morphological evolution displayed by a group of organisms. Whilst these "morphospecies" often do correspond to genuine biological or phylogenetic species, there is no *a priori* reason why they should automatically do so, particularly where the populations under comparison are in allopatry.

Unlike morphological data, molecular data allow a direct assessment of the genetic cohesiveness of populations by being able to examine a large number of homologous,

identifiable genes. Recent allozyme studies on a wide range of Australian vertebrates have revealed that perhaps more than 10% of morphospecies do not correspond directly with biological or phylogenetic species (Adams *et al.* 1987; Donnellan and Hutchinson 1990; Baverstock 1990; Adams, unpublished). A common finding from this allozyme work is that the number of morphospecies often underestimates the actual number of biological or phylogenetic species present. For convenience we refer to the extra taxa not identified using morphological criteria alone as cryptic species.

The Australian invertebrate groups have been comparatively little studied using allozyme techniques, but the work done to date suggests that there are much higher incidences of cryptic species in general than for the vertebrates (Riley *et al.* 1988; Horwitz *et al.* 1990; Chilton *et al.* 1992; Adams, unpublished). This situation is likely to be particularly serious in groups such as parasites and troglobites where there has been morphological de-differentiation, resulting in a general lack of taxonomic characters. With respect to troglobites, cryptic species have already been demonstrated amongst cave-dwelling millipedes using allozyme data (Laing *et al.* 1976a). Studies of this sort strongly point to the need for a molecular assessment of the genetic integrity of the common morphospecies present on Cape Range peninsula.

Group by group patterns of genetic diversity

We turn now to a group by group assessment of the allozyme data. To assist in coordinating this information, a series of tables summarizing the data for all groups is presented according to the component of the systematic hierarchy under examination. Table 3 focuses on species delineation by presenting an overview of the between-population genetic distances which characterize each group. In subsequent tables are collated the intraspecific genetic diversity

Table 3. Summary of the genetic distances between populations within six groups of cave-dwelling fauna on the Cape Range peninsula. Nei's genetic distance is calculated according to Nei (1978), whilst % fixed differences are described in Richardson *et al.* (1986). References for individual sets of allele frequencies: [A]Adams and Humphreys, unpublished, [B]Humphreys *et al.* 1989, [C]Humphreys and Adams 1991. *An approximate estimate, as the individuals representing this species were so small that samples had to be pooled in order to obtain sufficient material for analysis.

Genetic distances between populations		IsopodsA	Schizomids[B,A]	Millipedes[A]	Amphipods[C,A]	Shrimps[C,A]	Gudgeons[C]
1) all populations							
	ave	0.001	0.037	0.246	0.643	0.463	0.04
Nei D	min	0	0	0	0.033	0.005	0
	max	0.004	0.152	0.86	~1.4*	0.791	
Percent fixed	ave	0	2.4	15	38.3	29.4	2.3
differences	min	0	0	0	0	0	0
	max	0	9	50	70	50	7
2) within morphospecies				(N=1)	(N=1)	(N=2)	
	ave	-	-	0.219	0.181	0.034	-
Nei D	min	-	-	0	0.033	0.009	-
	max	-	-	0.526	0.31	0.073	-
Percent fixed	ave	-	-	13.4	11.3	1.5	-
differences	min	-	-	0	0	0	-
	max	-	-	35	23	6	-

(Table 4) and the information available on the reconstruction of evolutionary relationships within each group (Table 5). Where appropriate, dendrograms depicting the genetic similarities between populations and taxa within a group are also presented (Figures 2-6). Dendrograms offer a convenient way of depicting the broad genetic affinities of populations, although being one-dimensional representations of multi-dimensional data sets, they cannot accurately display all pairwise combinations of genetic distance. As they are constructed using a phenetic rather than a phylogenetic method of analysis, these dendrograms do not necessarily display the true evolutionary relationships between lineages (Richardson *et al.* 1986).

Isopods

The isopods were included primarily as a non-troglobitic reference, and were not intended to be a major group under study. Five populations from the central and southern provinces of Cape Range were examined for allozyme variation at 32 loci (Tables 1 and 2; Figure 1). The levels of genetic differentiation between populations were very low (0% fixed differences, Nei Ds 0.000 - 0.004, Table 3) and provided no indication that more than a single biological species was present. Moreover, there was no indication from the F-statistics of any significant departure from the expectations for panmixia within the species (Table 4).

Schizomids

Eighteen populations of *Draculoides vinei* were sampled from all three provinces of Cape Range and characterized for 23 loci (Tables 1 and 2; Figure 1). A total of 18 loci were invariant across all populations, resulting in only five loci being informative for genetic analysis. Most of the genetic diversity present in this group results from three loci where fixed differences are present between some populations. The pattern of genetic variation does not however indicate the presence of cryptic species, for two reasons. First, an examination of the allele frequencies reveals that no combination of two or more populations can be categorized by alleles not found elsewhere in the other populations sampled. Second, the genetic distance

Table 4. Population genetic parameters for taxa represented by more than one population site (the Barrow Island population has been excluded for *S. stylifera*.) The following taxa were represented by only a single population; millipedes - sp2 ($H = 0.030 \pm 0.024$), amphipods - northern province ($H = 0.083 \pm 0.031$), and amphipods - southern province ($H = 0.056 \pm 0.026$). Heterozygosity (H) was calculated as expected heterozygosity for populations under Hardy Weinberg equilibrium and averaged over all populations. F-statistics were calculated according to the rationale of Weir and Cockerham (1984) and using the computer program of Weir (1990). *Significant at the 5% level.

Taxon	Sites	Average level of H per population	Fixed diffs between populations?	F_{ST} Mean	F_{ST} 95% CI	F_{IS} Mean	F_{IS} 95%CI
Isopods	5	0.083 ± 0.032	No	0.008	(-0.046 to 0.046)	0.151	(-0.116 to 0.260)
Schizomids	18	0.012 ± 0.011	Yes	0.688	(0.538 to 0.960)*	0.163	(-0.083 to 0.246)
Millipedes-northern province	9	0.063 ± 0.027	Yes	0.397	(0.199 to 0.587)*	0.059	(-0.015 to 0.212)
Millipedes-central province	15	0.055 ± 0.029	Yes	0.466	(0.378 to 0.608)*	0.017	(-0.129 to 0.068)
Millipedes-southern province	12	0.035 ± 0.018	Yes	0.356	(0.051 to 0.597)*	-0.089	(-0.143 to 0.119)
Amphipods-central province	2	0.049 ± 0.026	No	0.324	(0.097 to 0.472)*	0.216	(-0.059 to 0.400)
Shrimps-*S. lancifera*	4	0.037 ± 0.021	No	0.324	(0.095 to 0.473)*	0.203	(-0.155 to 0.455)
Shrimps-*S. stylifera*	3	0.036 ± 0.018	No	-0.095	(-0.130 to 0.255)	0.221	(-0.121 to 0.160)
Gudgeons	7	0.028 ± 0.013	Yes	0.457	(0.211 to 0.599)*	0.208	(0.071 to 0.300)*

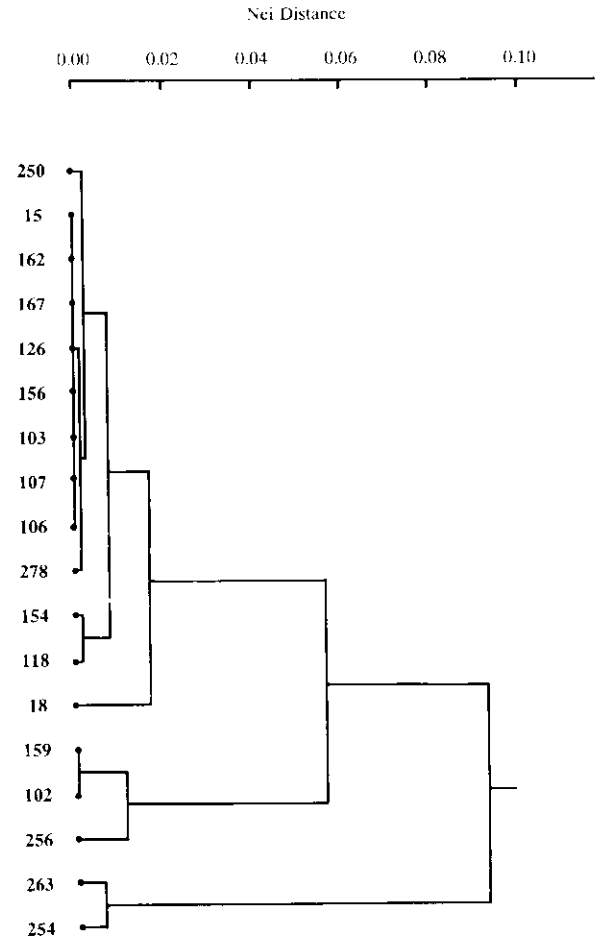

Figure 2. Dendrogram depicting the genetic relationships amongst the 18 populations of schizomids. All dendrograms presented (Figures 2-6) have been constructed using the Unweighted Pair Group Method of Analysis on the between-population genetic distances (Nei D).

values fall well within the range normally encountered for allopatric samples of the same species (Thorpe 1983).

The allozyme data reveal that the schizomids are highly substructured at the population level. The salient points here are the presence of fixed differences in combination with the large value of F_{ST} which is significantly greater than zero (Table 4). An examination of the dendrogram (Figure 2) shows that no simple geographic patterns exist for this species. Five of the populations differ by Nei D > 0.05 from remaining populations, and these represent sites scattered over two of the geographic provinces of Cape Range. In addition there is a genetically homogeneous cluster of populations comprising caves from all three provinces. To emphasize the absence of a geographic trend, the greatest level of divergence found between any two sites involves caves C-256 and C-263, which are adjacent to each other in the northern province (Figure 1). There is clearly no gene flow currently taking place between these two caves, and the data provide no support at all for the notion that frequent wholesale

subterranean dispersal takes place.

Turning to within-population matters, there is no indication that populations within a cave are not behaving panmictically, either from an examination of genotype frequencies or a consideration of the value of F_{IS} (Table 4). The average level of heterozygosity per population is the lowest encountered for any of the six groups on the Cape Range peninsula (Table 4).

Millipedes

This group has been the most intensively studied of all the subterranean fauna on Cape Range peninsula. A large number of populations have been sampled over several years and analyzed electrophoretically in two separate batches. The first part of the analysis looked at a selection of populations from across the range of the common morphospecies, *Stygiochiropus communis* and characterized these populations at 24 enzyme loci. In view of the high levels of genetic diversity encountered in this analysis, a large number of additional populations were further analyzed for the 18 loci found to be variable in the first part of the analysis. During the course of this second analysis, one cave (C-111) revealed the presence of two biological species which displayed fixed differences in sympatry at six out of 17 scorable loci. This additional biological species (*S. sympatricus*) was independently determined as a distinct morphospecies (along with another morphospecies, *S. isolatus*) and is known only from this single population (Humphreys and Shear 1993). *S. sympatricus* differs from all other populations by an average of 39% of loci examined (Figure 3, Table 5). The 36 populations of *S. communis* have been characterized at 18 loci; for the purposes of between-group comparison, all measures of genetic diversity have been calculated conservatively by assuming that the six monomorphic loci from the initial analysis remain monomorphic throughout all populations.

The millipedes displayed high levels of genetic variation, even after removing the additional

Table 5. Summary of the cladogenic events demonstrable within each group.

Group	Cladogenic event		Level of genetic divergence (Nei D)	(% fixed diffs)
Isopods	none demonstrable		-	-
Schizomids	differentiation of cave populations		0.04	2.4
Millipedes	(1) *S. communis* from *S. sympatricus*		0.6	38.9
	(2) southern province from the rest		0.33	22.4
	(3) central province from northern province		0.24	12.4
	(4) differentiation of cave populations	- northern	0.07	2.2
		- central	0.06	1.6
		- southern	0.02	0.6
Amphipods	(1) sp1 from sp2		~1.4	70
	(2) southern province from the rest		0.26	18.3
	(3) central province from northern province		0.13	6.5
	(4) differentiation of central cave populations		0.03	0
Shrimps	(1) *S. lancifera* from *S. stylifera*		0.75	50
	(2) differentiation of populations	- *S. lancifera*	0.04	2
		- *S. stylifera*	0.08	5.6
Gudgeons	differentiation of populations		0.04	2.3

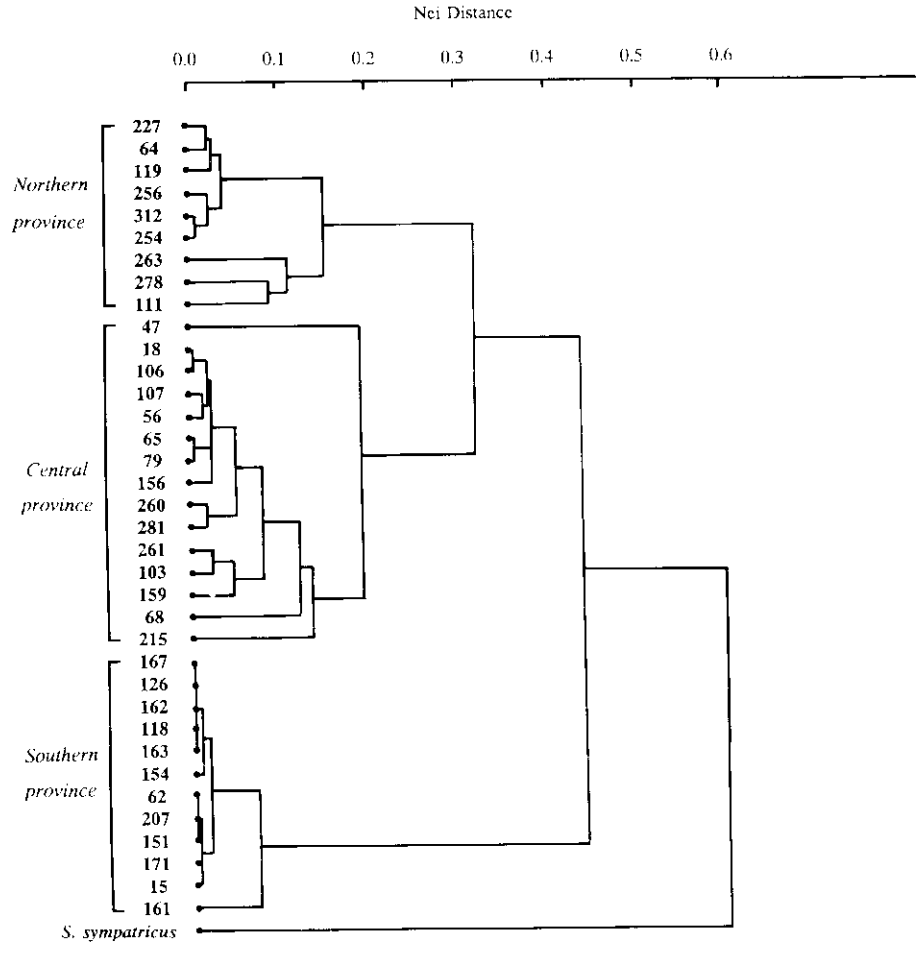

Figure 3. Dendrogram depicting the genetic relationships amongst the 36 populations of the millipede *Stygiochiropus communis* and including the single population of *S. sympatricus*. The genetic distances have been generated using only 18 of the 24 loci originally identified.

diversity caused by the inclusion of *S. sympatricus* (Table 3). Populations fall into three distinct clusters that delineate the three geographic provinces of Cape Range (Figure 3; see Humphreys 1993). Each province is characterized by the presence of at least two fixed differences when compared to the other two provinces. The southern province in particular is distinguishable from the others by fixed differences involving unique alleles at three loci. The levels of genetic divergence encountered between the provinces (Figure 3, Table 5) are not sufficient in allopatry to demonstrate the presence of more than one biological species without reference to additional systematic data. However, the application of a strict phylogenetic species concept would recognize the existence of at least two and possibly three phylogenetic species. Whilst we do not intend to refer to the three provinces as separate species (cf. Humphreys and Shear 1993), it is nevertheless appropriate to regard them as separate taxa for the purposes of more detailed within-group analysis.

An analysis of geographic differentiation within each of the three provinces indicates that population substructuring is occurring in all three cases. All three provinces show significantly positive values for F_{ST} and display instances of fixed allozymic differences between cave populations (Table 4). The southern province shows the least amount of differentiation between populations, with much of this differentiation being attributable to a series of divergent allele frequencies in cave C-161 (Figure 1). This cave does not share any alleles in common with an adjacent cave (C-15, Figure 1) at one locus, demonstrating that there is currently no gene flow between the two populations, which lie respectively in westward and eastward draining catchments. Reanalysis of F-statistics for the southern province with cave C-161 taken out reveals that the remaining populations continue to show a marginally significant divergence from overall panmixia (F_{ST} = 0.223; 95% confidence intervals = 0.040 - 0.297). The northern province shows greater levels of divergence between cave populations, with fixed differences characterizing a number of pairwise comparisons of populations, and a value of F_{ST} that cannot be made non-significantly different from zero by removing one or two populations from the analysis (Table 4). To take a specific example, the adjacent caves C-263 and C-256 (Figure 1) have significantly different allele frequencies at no less than five loci, demonstrating that there is currently no gene flow between the two caves; these caves lie on opposite sides of a small gorge. The central province presents the greatest levels of genetic differentiation, with fixed differences common between cave populations, and a highly significant value for F_{ST} (Table 4). Whilst there appears to be a suggestion of some microgeographic pattern to the distribution of allele frequencies, the small sample sizes per population preclude any detailed analysis. Summarizing, the allozyme data confirm the presence of population substructuring within all three provinces and indicate that gene flow is limited within any province.

With respect to intra-population variability, there was no indication from either genotype frequencies or F_{IS} values that the animals in any cave were violating the assumption of random mating (Table 4). Levels of average heterozygosity were comparable between the northern and central provinces, but were somewhat reduced in the southern province, reflecting fewer polymorphic loci in this region (Table 4).

Phylogenetic analyses of the allozyme data using both the "Distance Wagner Method" (Farris 1972) and Swofford's parsimony analysis of allele frequencies "FREQPARS" (Swofford and Berlocher 1987) clearly support the basic topology of the tree shown in Figure 3. The main features of this phylogeny are the monophyly of all populations of *S. communis* to the exclusion of *S. sympatricus*, and the monophyly of the central and northern provinces to the exclusion of the southern province. The levels of genetic distance that characterize these cladogenic events are given in Table 5.

Amphipods

Only four caves on Cape Range present a suitable habitat for this aquatic group (Table 2). These caves are spread out over the three geographic provinces, with two populations located within the central province (Figure 1). An additional population representing another morphospecies present on the coastal plain was also available for genetic characterization at 21 of the 34 loci run for the group as a whole. A dendrogram depicting the genetic relationships between populations is presented in Figure 4. The most obvious feature of the dendrogram is the high level of genetic divergence between the two morphospecies of amphipod. The values obtained (Nei D ~ 1.4; 70% fixed differences, Table 5) are the largest for any group in this study.

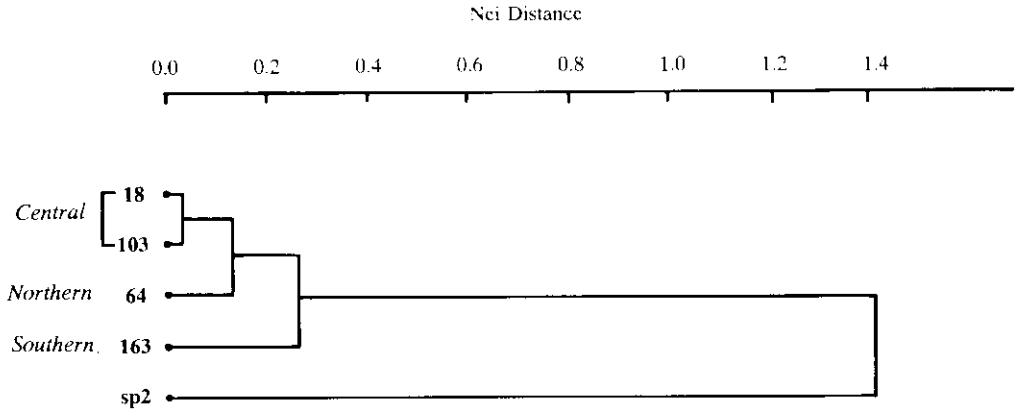

Figure 4. Dendrogram depicting the genetic relationships amongst the four populations of the amphipods from Cape Range and including morphospecies sp2.

Within the morphospecies on Cape Range, a pattern of regional differentiation emerges which is similar to that displayed by the millipedes. Three clusters are evident from an analysis of % fixed differences (a figure not shown, but the topology differs from Figure 4 only in that caves C-18 and C-103 have 0% fixed differences), and these again correspond to the northern, central and southern provinces. Furthermore, the genetic relationships among the three provinces are the same, with the southern province being the most divergent of the three. As with the millipedes, the population in the southern province is characterized by fixed differences for unique alleles at three loci. Phylogenetic rather than phenetic analyses of the allozyme data support this arrangement of the provinces. Once again, we have chosen to view the three provinces as representing several taxa, without forming an opinion as to their relative taxonomic ranks.

As the one province containing two populations, only the central province can be assessed for within-province differentiation. Although no fixed differences were present between the two sites, the F_{ST} value was significantly greater than 0, indicating that substructuring can be detected (Table 4). All populations displayed genotype frequencies compatible with Hardy Weinberg expectations, and F_{IS} was not significantly greater than zero for the central province (Table 4). Finally, the average levels of heterozygosity for both central and southern provinces were comparable, with the northern site having the equal highest level for all faunal groups studied (Table 4).

Shrimps

Two species of *Stygiocaris*, sympatric through part of their range, have been described from the coastal plain (Holthuis 1960). Shrimps from eight populations, including a sample from Barrow Island, were surveyed for allozyme variation at 30 loci (Tables 1 and 2, Figure 1). The results do support the recognition of two species within the localities sampled; these species differ at about 50% of loci examined (Table 5). The two species have overlapping geographic distributions, with *S. lancifera* found on the west coast of the Cape Range peninsula, *S. stylifera* found mainly on the east coast and on Barrow Island, and both species present in one well (C-25) at the northern end of the west coast (Figure 1).

A dendrogram depicting the genetic relationships amongst all populations of shrimps is presented in Figure 5. Within *S. lancifera*, populations are genetically very similar, but

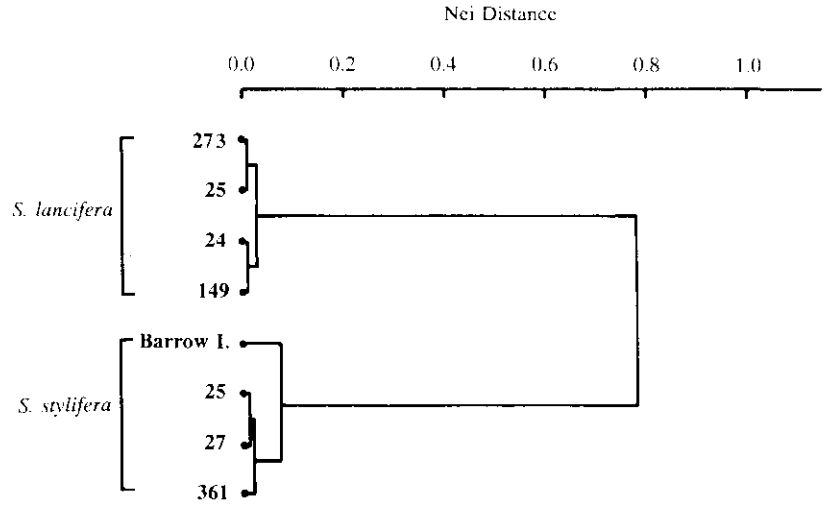

Figure 5. Dendrogram depicting the genetic relationships amongst the eight populations of shrimps.

nevertheless show a sufficient level of genetic divergence to be discernable using F-statistics (Table 4). Within *S. stylifera*, the Barrow Island sample was fixed for a unique allele at one of the 18 loci examined (Table 5). After excluding this population, no significant indications of substructuring are evident within the remaining three populations on Cape Range peninsula using F-statistics (Table 4). No populations in either species showed evidence of departures from Hardy Weinberg expectations, and average levels of heterozygosity were similar and at the low end of the scale for the six groups (Table 4).

Gudgeons

Seven populations of *Milyeringa veritas*, distributed all around the edge of the coastal plain, were subjected to a comprehensive allozyme analysis at 43 loci (Tables 1 and 2, Figure 1). Whilst some fixed differences were encountered, the overall levels of genetic differentiation between populations and the geographic pattern of allele distribution are both consistent with

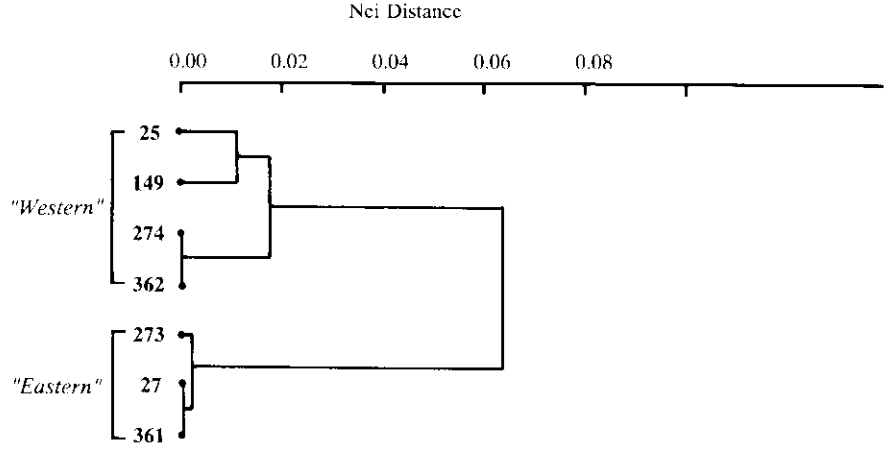

Figure 6. Dendrogram depicting the genetic relationships amongst the seven populations of gudgeons.

the presence of a single biological species (Table 3, Figure 6). An analysis of F-statistics demonstrates the presence of significant population substructuring in this species (Table 4).

The genetic relationships amongst populations are displayed visually in Figure 6. Two major clusters are evident and these reflect a general "western" versus "eastern" geographic split. Populations C-27 and C-361 from the east coast of Cape Range peninsula are invariant at all loci examined, and display 5-7% fixed differences when compared to all but one of the populations on the west coast. The exception is the geographically intermediate well C-273 (Figure 1) at the northern end of the peninsula, which is genetically intermediate between the two groupings. This within-species geographic trend broadly mimics the distribution of the two species of shrimp, which overlap in C-24, C-25, and C-273 (Humphreys and Adams 1991).

The average level of heterozygosity in the gudgeons was the lowest encountered in any of the taxa examined (Table 4), and may reflect a commonly observed difference between vertebrates and invertebrates (Nevo *et al.* 1984). However, the existence of two populations for which $H = 0$ has contributed significantly to this low value. Interestingly, the gudgeons are the only group in which the F-statistics suggest there may not be random mating occurring within subpopulations (Table 4). It is not possible to explore the significance of this result any further without additional sampling.

Patterns of genetic diversity

We now turn to a search across all groups for general patterns of genetic diversity, trying to identify any biogeographic implications which may underlie such patterns, and beginning with a consideration of what, if any, conclusions can be drawn from other comparable studies on cave-dwelling organisms.

Other studies on cave-dwelling fauna

A search of the literature indicates that there are only a few studies addressing genetic diversity in cave-dwelling groups. Allozyme studies have been published on cave crickets (Cockley *et al.* 1977; Sbordoni *et al.* 1981; Caccone 1985), cave beetles (Laing *et al.* 1976b; Turanchick and Kane 1979; Delay *et al.* 1980; Brunner and Kane 1981; Crouau-Roy 1984, 1987), cave spiders (Cesaroni *et al.* 1981), amphipods (Dickson *et al.* 1979; Gooch and Hetrick 1979; Kane *et al.* 1992), cave millipedes (Laing *et al.* 1976a), and cave-dwelling fishes (Avise and Selander 1972; Swofford *et al.* 1980). Much of this allozyme work has been directed towards documenting comparative levels of genetic variability (H) in troglobitic versus non-troglobitic fauna, although more recent work has used allozyme data to examine processes of evolution in cave taxa (Wilkens 1988; Kane and Culver 1992). While different hypotheses predict that H in troglobites should be lower or higher than in epigean taxa, no consistent trend is evident (Culver 1982).

Apart from assessing comparative levels of variability, these studies have also shown that troglobites exhibit widely ranging levels of genetic divergence between populations, depending upon the predicted time of isolation, the potential for gene flow between localities, and the number of cryptic species present in the sample. The picture that emerges is that each combination of area and taxon has its own specific evolutionary history.

Most work on the systematics and zoogeography of cave systems has concentrated on the evolutionary history of single lineages or species swarms associated with the isolation of cave populations (e.g. Peck 1981; Crouau-Roy 1984, 1987). In no case has a series of different taxa occupying the same cave systems been jointly assessed from systematic and biogeographic

perspectives. Thus there are no relevant studies available which look at overall patterns of genetic diversity for a single microgeographic area.

Phylogenetic diversity

We use the term phylogenetic diversity here as a concept encompassing both the number of divergence events demonstrable within a group and the relative "ages" of these events, as estimated by the extent of genetic divergence. A diagrammatic representation of the extent of phylogenetic diversity within each group is presented in Figure 7, based on the genetic distances of Table 5. Such a presentation is not intended to imply that cladogenic events in different groups are necessarily directly assessable on a single time scale. This would only be the case if rates of molecular evolution are the same in all lineages. However, in the complete absence of any other relevant biological information, this comparative framework can serve as a null hypothesis for future assessment (Humphreys 1993).

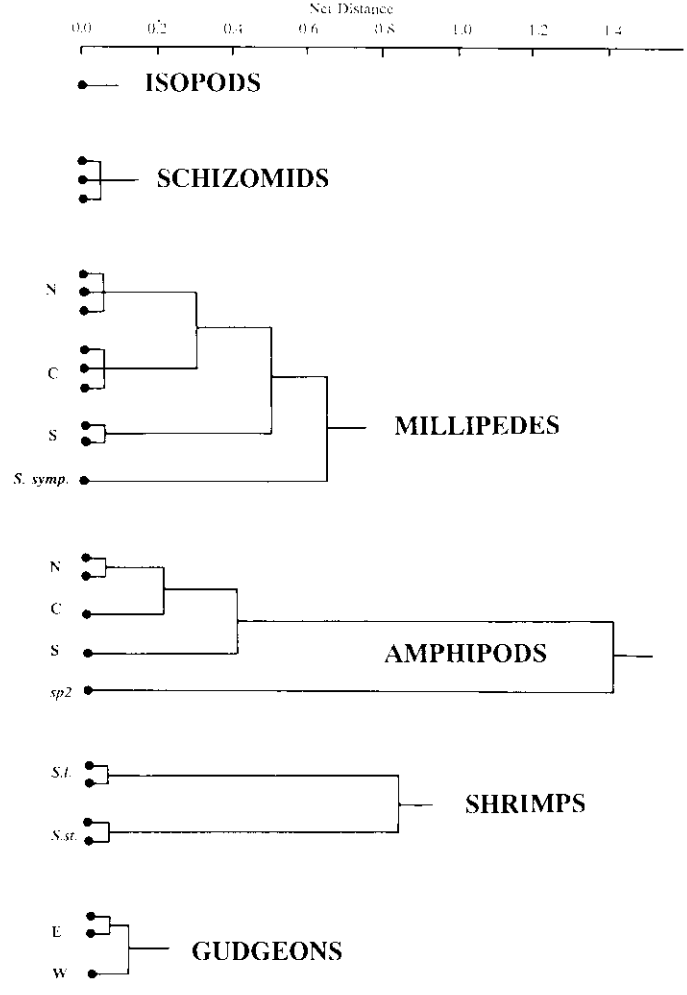

Figure 7. Diagrammatic representation of the cladogenic events which have occurred within each group as identified by the allozyme data shown in Table 5.

As shown in Figure 7, there is considerable disparity in the degree of phylogenetic diversity displayed within the six different groups. This conclusion persists even after the removal of the contribution due to the presence of extra morphospecies in some groups. The millipedes and the amphipods both show two additional hierarchical levels within their systematic frameworks when compared to the other morphospecies, whilst only the non-troglobitic isopods show no population differentiation. Knowledge of this situation changes the biogeographic perspectives on taxa which would otherwise have to be regarded as equivalent in terms of temporal and geographic differentiation.

Geographic patterns

Some indication of a geographic pattern emerges within each of the two major regions, namely the coastal plain and Cape Range itself. Whilst these within-region patterns are not robust enough to stand alone as biogeographic observations, they should nevertheless be addressed as issues against which other data sets can be examined for concordance. There is evidence of a discontinuity between the "eastern" and "western" sides of the coastal plain, both in the relative distributions of the two species of shrimp and the geographic patterning of genetic divergence in the gudgeons. On Cape Range, the concordance between the distribution of the three geographic provinces and presence of deep gorges is a matter for some consideration, particularly since only two of the three groups examined follow this pattern. The schizomids show no simple geographic substructuring, a result that may point to a more recent adaptation to cave dwelling, historically higher levels of gene flow between regions, or a major slowing down of the rate of biochemical evolution in this lineage.

Gene flow

The sample sizes available for most populations are inadequate to undertake any comprehensive analysis of gene flow using private alleles or F-statistics (Slatkin 1987). Our treatment of this aspect of intraspecific diversity has been limited in this study to an examination for any obvious trends. The non-troglobitic isopods show no significant genetic divergence between sampled populations, indicating that our data cannot rule out free gene flow between populations. Of course this in no way proves that gene flow exists in this species, as the probability of a type II error is very high given the sample sizes involved (Baverstock and Moritz 1990). Turning to the troglobites, most taxa examined show highly significant population substructuring when compared to the expectations of a single panmictic species (Table 4). Moreover, the existence of fixed allozyme differences between certain adjacent populations in most groups demonstrates that there is currently no gene flow between the localities so characterized. Whether this lack of gene flow extends to all other combinations of populations within each taxon or whether gene flow occurs between certain caves via some system of subterranean corridors cannot be addressed by this set of allozyme data.

Within-species genetic variability

There was some indication that the troglobitic species were genetically less variable than was the non-troglobitic control. Taken as a whole the 11 troglobitic taxa do show somewhat reduced values for H, although the large variation in the values obtained (range 0.028 to 0.083) serves to emphasize the lack of any clear differences. The values encountered for all taxa fall well within the range commonly encountered for vertebrates and for the non-social invertebrates (Nevo *et al.* 1984; Sherwin and Murray 1990). Moreover, whilst the isopods had

the equal highest level of genetic variability, the single population of the "northern-province" amphipod also had the same high level (Table 4). The lack of any clear trends in this study mirrors the findings of some other analyses (Culver 1982).

Overall patterns of biodiversity

No simple ecological or phylogenetic patterns of genetic diversity emerge in any comparison between the six groups. There are no unambiguous trends evident in the extent of heterozygosity or genetic distance between vertebrate and invertebrate, terrestrial and aquatic, or troglobitic and non-troglobitic fauna. This is in line with broad expectations, as the general conclusion from other studies is that so many species-specific factors influence the way genetic diversity is distributed within and between species that simple trends would not be likely to exist in any comprehensive comparison between different taxa.

Conclusions

In summary, we believe that there are three major conclusions to be drawn from the allozyme studies outlined in this paper. These are:-

1. A robust systematic framework, independent of any considerations of morphology, has been provided for all six faunal groups. This framework includes both the identification of major species and population lineages, and the sequence under which taxa have diverged within a group. The allozyme data also provide information regarding the relative age of divergence events in five of the six groups.

2. Two major geographic trends are evident on Cape Range peninsula, namely the existence of separate provinces within Cape Range for two of the three morphospecies examined, and a suggestion of an "eastern" versus "western" split near the top of the peninsula for the fauna occupying the coastal plain.

3. The allozyme data confirm the presence of substantial population substructuring within most troglobitic species, and infer that high levels of gene flow are not characteristic of geographically-adjacent caves. In contrast, the allozyme data for the one non-troglobitic group were consistent with the existence of some gene flow between cave populations.

We believe that this study serves as a demonstration of the relevance of molecular techniques in providing useful systematic perspectives in any situation where detailed biogeographic study is to be undertaken.

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