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Remane´s statistic species criterion
applied to Hawaiian cave planthoppers
(Hemiptera: Auchenorrhyncha: Fulgoromorpha: Cixiidae)

With 6 Figures and 2 Tables

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Abstract. Among populations of *Oliarus polyphemus* Fennah, a troglobitic species found in numerous lava tubes on Hawaii Island, a high degree of behavioral differentiation concerning the intraspecific communication signals has been observed (Hoch & Howarth 1993). Hoch & Howarth (1993) hypothesized that some of these populations were likely to represent species in *statu nascendi* or even reproductively isolated species. In order to qualitatively and quantitatively survey the discontinuities between the populations variance and discriminance analyses were carried out based on morphological and ethological characters of individuals from 10 caves. According to the statistic species criterion as proposed by R. Remane (1968), nine populations must be regarded at least as subspecies, while one population is likely to have acquired species status.

Introduction

The discovery of obligately cavernicolous (troglobitic) arthropods in lavatubes on geologically young oceanic islands by F.G. Howarth on Hawaii (Howarth 1972) challenged more conventional theories on the evolution of cavernicolous organisms: Howarth (1986) sees the evolutionary process of cave-adaptation as an active adaptive shift to novel habitats rather than a reaction to unfavorable ecological conditions on the surface (e.g., glaciation) resulting in the extinction or extirpation of surface populations. The observation that in Hawaii, close epigean relatives of obligately cavernicolous species occur parapatrically in surface habitats on the same island supports this view. In Hawaii, the taxon *Oliarus* Stål is represented with ca. 80 species in the epigean fauna of all major islands. Morphological information supports the hypothesis that all Hawaiian *Oliarus* species are monophyletic (Asche 1997), i.e. descendants of a single successful colonizing species. Within this group, at least seven separate evolutionary lineages have colonized the subterreanean biome: one on Molokai, three on Maui, and at least three on Hawaii Island (Hoch & Howarth in press). One of these lineages, *Oliarus polyphemus* (Fennah 1973) from Hawaii Island is a morphologically highly modified, blind, flight- and pigmentless species. It had been regarded a widespread cave species on Hawaii Island since populations were known to exist in numerous lava tubes of Mauna Loa, Kilauea, Mauna Kea, and Hualalai volcanoes. Earlier investigations of their intraspecific communication revealed that cavernicolous Fulgoromorpha retain the same mode of communication as their epigean relatives: they communicate via substrate-borne vibrations (Howarth et al. 1990). In the cave environment, roots of native Hawaiian plants, especially *Metrosideros polymorpha* (Myrtaceae) on which the planthoppers feed, provide an excellent substrate for the transmission of vibratory communication signals (Hoch & Howarth 1993).

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A comparative study of mating behavior within and among *O. polyphemus* populations from different lava tubes revealed comparatively little variation with single populations; a high degree of variation among populations from different lava tubes, however, was observed (HOCH & HOWARTH 1993). As courtship songs play a pivotal role in species recognition as has been shown in epigean planthoppers (CLARIDGE 1990, HENRY 1994, STRÜBING 1977) and have highly species-specific patterns, it appeared plausible to assume the existence of a complex of *"cryptic acoustic species"* (HOCH & HOWARTH 1993). The young geological age of Hawaii Island (about 500,000 years) (MCDONALD & ABBOTT 1970) and the small degree of morphological differentiation pointed towards a young species complex in a dynamic stage of its development (HOCH & HOWARTH 1993).

The present study combines the analysis of morphometric information with the results of courtship song analysis in HOCH & HOWARTH (1993) and additional new data in order to assess in more detail the degree of morphological and ethological variation within and among the populations studied. In order to use these data for judgements on the species status of a population we applied R. REMANE's criterion (REMANE 1968): he postulates that the presence or absence of gene flow between two populations will be expressed in the phenotype. According to Remane (l.c.), morphological discontinuity between populations is likely to be an indication for the absence of gene flow. If the ranges of variation of at least one non-modifiable character do not overlap in at least one sex, the populations compared can be regarded as reproductively isolated entities, i.e. separate species. If the ranges of variation do overlap, but a significant separation of the populations by statistics is possible, they are considered to represent subspecies (REMANE 1968). REMANE's criterion thus allows to build a hypothesis on the species status of compared populations even if there is no possibility (yet) to apply the biospecies concept directly, e.g. by cross-breeding experiments. Here, we attempt to expand REMANE's criterion which was originally developed for structural characters to behavioral characters (song parameters) which play an important role in specific mate recognition. By doing this, we try to bridge the gap between the Biological Species Concept sensu MAYR (1940) and the Recognition Species Concept (PATERSON 1985).

Material and methods

Populations of *O. polyphemus* are known from 26 caves of four volcanic systems on Hawaii Island (Mauna Loa, Kilauea, Mauna Kea, Hualalai) (Fig. 1). For the analysis of the intraspecific communication signals a sufficient number of recordings was available from 10 populations (Tab. 1). Recordings were made using the magneto-dynamic system (STRÜBING & ROLLENHAGEN 1988) under field conditions as well as in the laboratory facilities at Bishop Museum, Honolulu, and Humboldt-University, Museum für Naturkunde, Berlin between 1989 and 1998 (for methods see also HOCH & HOWARTH 1993). The signals were digitized and measured with the program Chart v 3.5.4./s on the hardware/software unit Mac Lab/4s from ADInstruments running on a Power Macintosh 7600/132. The signals were recorded with a resolution of 40.000 data points per second and measured with an accuracy of 0.15 ms.

10 parameters of the time pattern of single calls (composed of more or less homogenous pulse trains [Fig. 2]) were taken (Tab. 2). Parameters 4 to 10 were included in the analysis as parameters 11–17 after omission of the first and last three pulses of a call in order to assess intraindividual variability (Fig. 3).

For the morphometric analysis, individuals from the same 10 populations were studied (Tab. 1). 9 parameters and 4 indexes computed from them were taken (Tab. 2). Measurements were made using a measuring ocular with an Olympus SZH 10 at 50-fold magnification (accuracy ±10 µm).

The 10 populations yielded a sufficiently large random sample, and data in both character complexes at least in one sex were used in the final discriminance analysis. Morphological and ethological characters were analyzed independently.

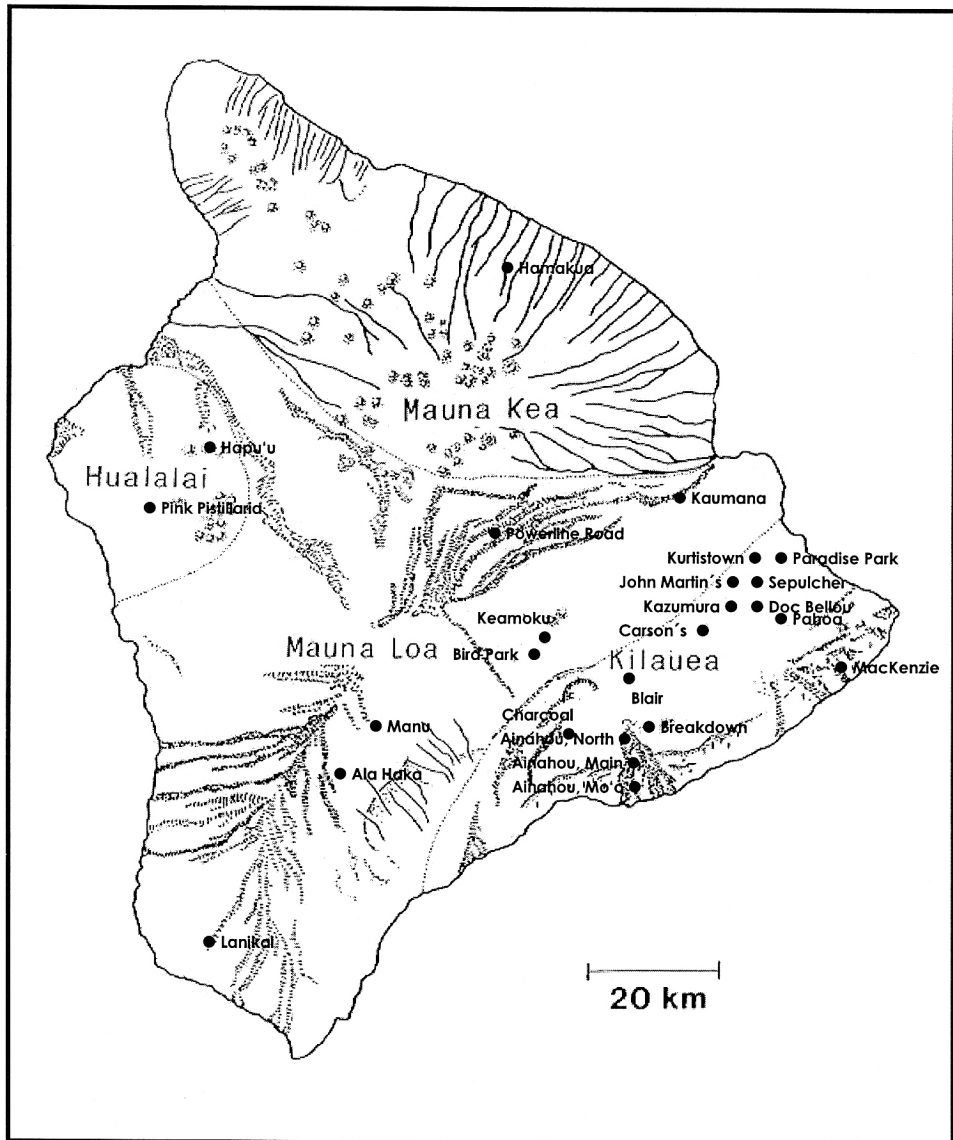For the statistic evaluation SPSS for Windows, release 7.0 was used.

Fig. 1: Location of study sites: map showing historic lava flows on Hawaii Island (redrawn from ARMSTRONG 1983).

Results

1. An unifactorial analysis of variance showed the discontinuities between the populations for all parameters to be highly significant ($P \leq 0.001$). The call-parameters discriminated higher (F-values between 22.6 and 157.8) than the morphometric parameters (F-values between 2.2 and 16.4).

2. A discriminance analysis between the sexes of each population revealed a distinct sexual dimorphism in both character complexes. Therefore, the sexes were analysed separately. Hence, in an

Tab. 1: Origin of material included in the statistic analyses.

	Morphometry		Songs			
	Individuals		Single calls (Individuals)			
	♀	♂	♀		♂	
Mauna Loa						
Kaumana Cave	15	28	53	(4)	44	(7)
Lanikai Cave	11	8	10	(1)	5	(1)
Kilauea						
Pahoa Cave	23	19	51	(4)	17	(4)
Ainahou Cave	18	25	10	(2)	15	(3)
Sepulcher Cave	9	17	5	(1)	5	(1)
Paradise Park Cave	11	17	10	(2)	7	(2)
MacKenzie Park Cave	15	12	30	(2)	10	(2)
DocBellou Cave	6	6	10	(2)	5	(1)
Blair Cave	5	3	22	(2)	-	-
John Martins Cave	4	15	-	-	8	(1)
	117	150	201	(20)	130	(22)

Tab. 2: Song parameters and morphological characters used in the statistical analyses.

| Song parameters		
01	Number of pulses per call	
02	Call duration	
03	Duration of the following pause	
04;11	Longest interpuls intervall (IPI)	
05;12	Shortest IPI	
06;13	Mean value of the duration of the IPIs	
07;14	Variation range of the IPI durations	
08;15	Standard deviation of the variation of the IPI durations	
09;16	Ascent of the linear regression above the sequel of IPI durations	
10;17	Intersection of the linear regression with the ordinate	
Morphological characters		
18	Wing length	
19	Wing width	
20	Pronotum length	
21	Mesonotum length	
22	Mesonotum width	
23	Head width	
24	Vertex width	
25	Length of middle tibia	
26	Length of rear tibia	
27	Wing proportion	= wing length:wing width
28	Relative wing length	= wing length:mesonotum width
29	Relative length of rear tibia	= length of rear tibia:mesonotum width
30	Head proportion	= vertex width:head width
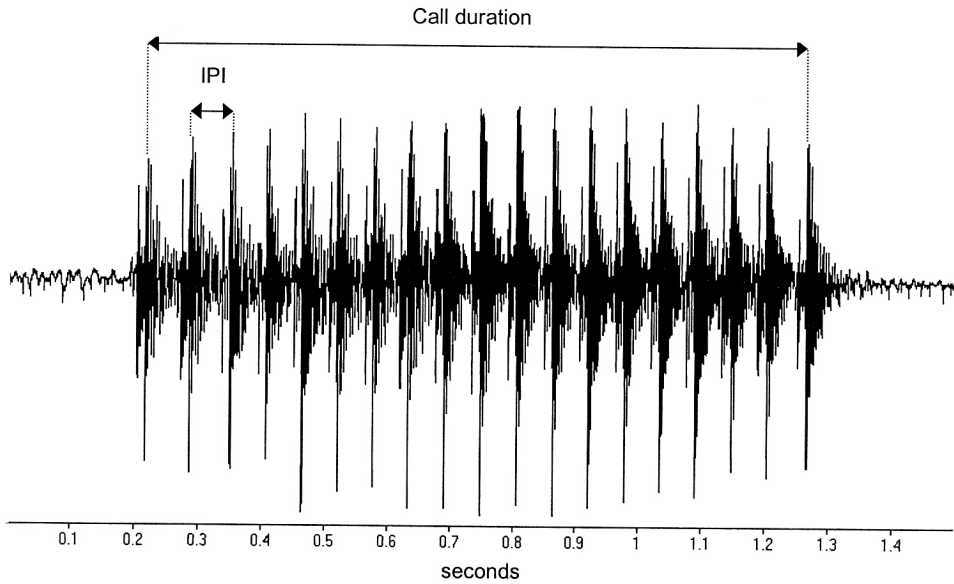
Fig. 2: Call structure of an *Oliarus polyphemus* female from MacKenzie Park Cave. (IPI = interpulse interval).
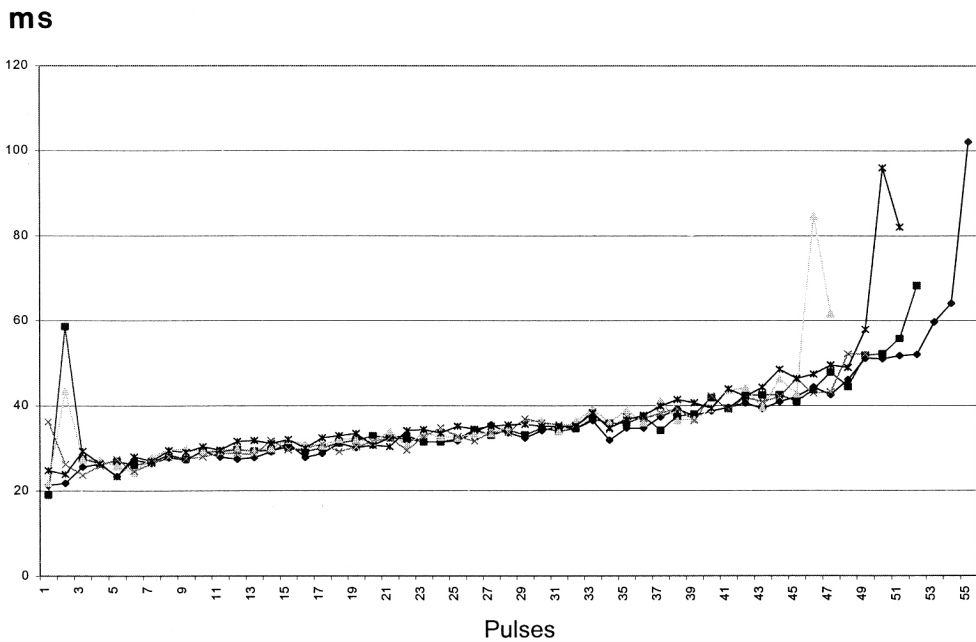
ms

Fig. 3: Intraindividual variation of interpulse interval duration within five single calls of a female from Paradise Park Cave.
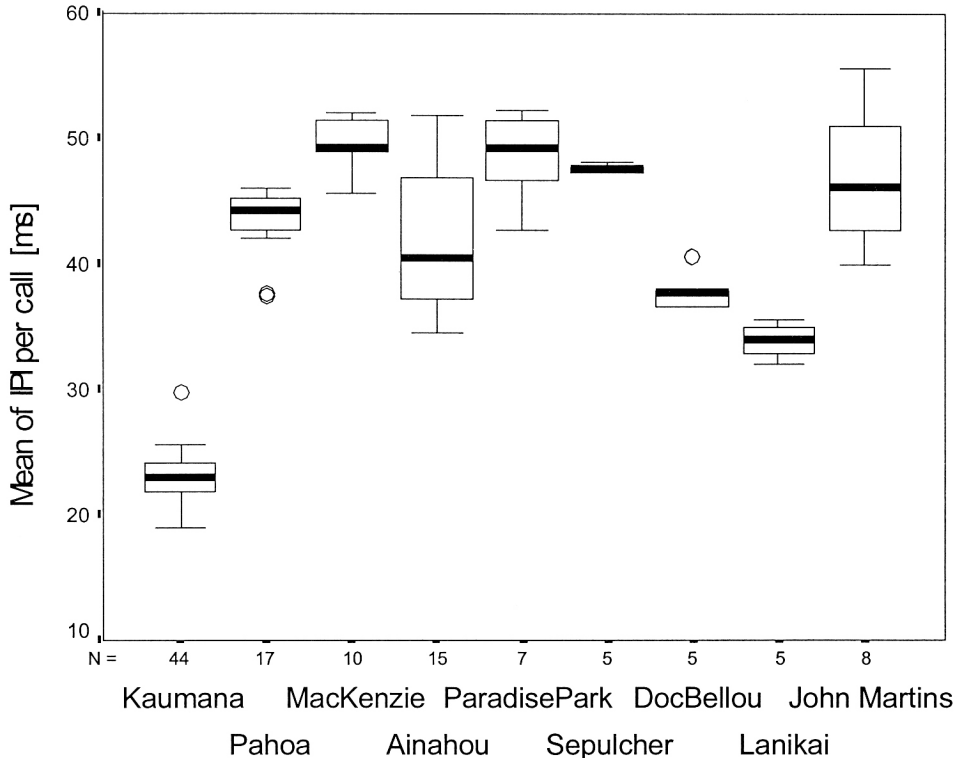
Fig. 4: Male call variation in the mean values of interpulse intervals (IPIs) per call.

analysis of variance for separated sexes the spread within the groups decreases strongly for each para-meter. If some F-values are still smaller than in the combined analysis it is due to the even stronger decreases of the spreads of the group mean values. Earlier studies showed, that two populations (Pahoa and Kaumana Cave) do not to overlap in the variation ranges of a character in both sexes for the call parameters (HOCH & HOWARTH 1993). These results could be confirmed, but the gaps were filled in by other populations (Fig. 4).

3. Both character complexes showed that the females were separated more significantly and are thus more differentiated than the males. The results of the discriminance analysis for the females in corre-lation with the geographic distribution are shown in Fig. 5 and 6.

4. One character, the mean value of interpulse interval duration (IPI duration), was found to show no overlap between the male individuals from Kaumana Cave population and all other populations (Fig. 4).

Discussion

The differentiation observed among *Oliarus polyphemus* populations raises the question about the systematic status of the single populations. We are faced with the problem of the applicability of the existing species concepts. If it is – as in most cases – not possible to apply the biospecies concept
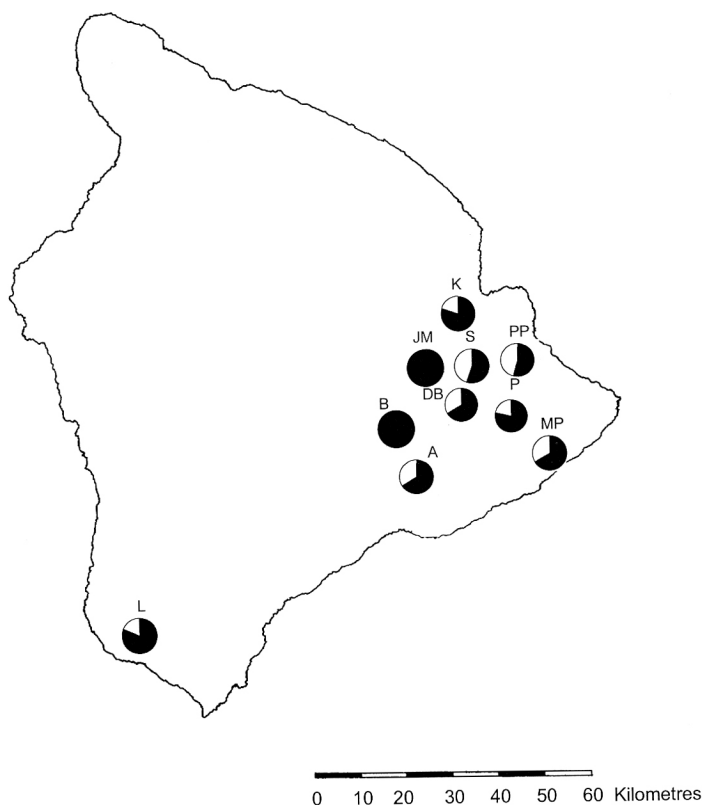
Fig. 5: Results of the discriminant analysis for the morphological characters for the female individuals from 10 caves. The black sections of the circles represent the correctly classified individuals. L, Lanikai Cave; B, Blair Cave; A, Ainahou Cave (North Entr.); JM, John Martins Cave; DB, DocBellou Cave; K, Kaumana Cave; S, Sepulcher Cave; P, Pahoa Cave; PP, Paradise Park Cave; MP, MacKenzie Park Cave.

(MAYR 1940) directly, a statistical analysis may reveal the degree of phenotypic differentiation and thus could enable us to infer the existence of biological meaningful units on the species level (compare MAYR & ASHLOCK 1991). This line of reasoning requires a theoretically sound criterion, though, to translate the degree of differentiation into a usable, i.e. falsifiable hypothesis on the species status of closely related populations. Following R. REMANE in the assumption that absence of gene flow between compared populations (sooner or later) establishes itself phenotypically, we can regard populations that show overlap in their ranges of variation, but can be (statistically) significantly discriminated as subspecies, in case of non-overlap in at least one character as separate species (REMANE 1968).

In this concept *any* measurable character is equally informative (sexual dimorphism, ontogenetic stages and polyphenism taken into account) when it comes to evidencing the actual absence of gene flow between closely related populations.

The term *"subspecies"* which is often used in the sense of *"populations of doubtful rank"* (MAYR & ASHLOCK 1991: 105) could thus be objectified by statistically describing the degree of genetic separation. The ranking of populations remains a working hypothesis, as any assignment of species status based on morphological characters. A hypothesis erected by application of the statistic criterion described above is falsifiable, though, in contrast to hypotheses gained by other "intuitive" ranking procedures.

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0   10   20   30   40   50   60  Kilometres
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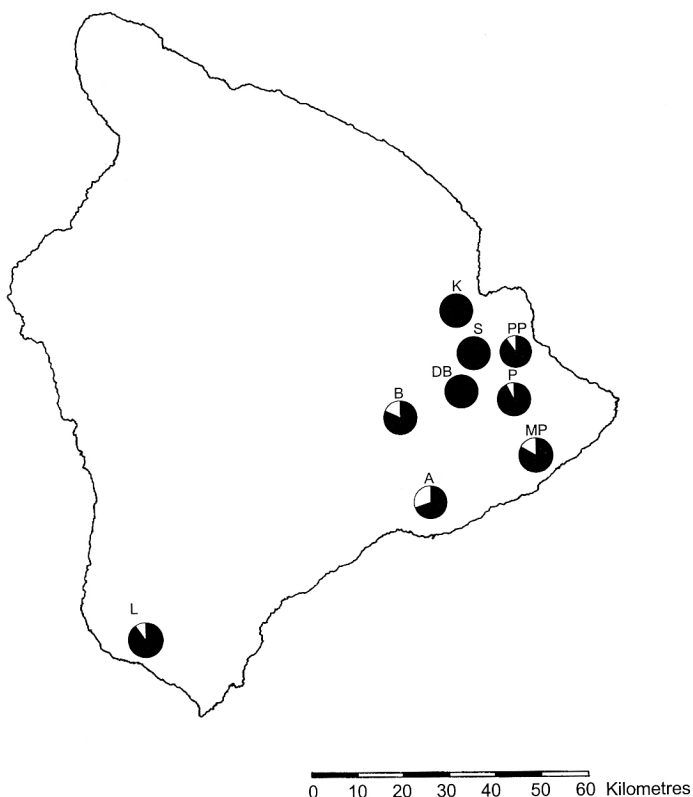
Fig. 6: Results of the discriminant analysis for the ethological characters for the female individuals from nine caves. The black sections of the circles represent the correctly classified individuals. L, Lanikai Cave; B, Blair Cave; A, Ainahou Cave (North Entr.); DB, DocBellou Cave; K, Kaumana Cave; S, Sepulcher Cave; P, Pahoa Cave; PP, Paradise Park Cave; MP, MacKenzie Park Cave.

The use of a non-overlap of variation ranges of any structural character by REMANE (1968) as a criterion for species status could be seen as drawing an arbitrary line. But this point of view neglects two important facts, the first merely being the existence of a clearly defined falsifiable hypothesis in this case as well. Secondly, a fundamental biological meaning – reproductive isolation – is implicit in regarding the non-overlap as a phenotypic hint for a beginning or existing genetic incompatibility.

By interpreting 9 populations of *O. polyphemus* as subspecies and the one from Kaumana Cave as a taxon that has acquired species status, we are indeed faced with a complex of species and subspecies instead of one widespread species as has been hypothesized by HOCH & HOWARTH (1993). This complex which comprises populations of varying age and degree of differentiation provides an excellent model to test the assumptions presented above and to evaluate the applicational limits for REMANE'S criterion when drawing conclusions on the inferred biological meaning.

The role of behaviour as a factor in evolution is especially interesting in this context. Among the populations studied, ethological characters (courtship song parameters) which are critical for specific mate recognition and thus for maintaining the populations' gene pool, differ significantly. This might indicate that reproductive isolation between the compared populations is far more progressed than is (yet) established morphologically. Historical geology allows for precise determination of cave age and thus

a possibility to reconstruct patterns of migration. The fact that the most distinct population which is now regarded a separate species occurs in one of the youngest caves (Kaumana Cave, in a Mauna Loa lava flow of 1881) might be explained by founder effects. Thus, with studying the *Oliarus polyphemus* species complex, we might be blessed with "… *a great deal of good fortune to encounter dynamic evolution of a biocommunicative system in natural populations*" (LITTLEJOHN 1988).

Acknowledgements

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