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## A BEHAVIOURAL, MOLECULAR AND MORPHOMETRIC APPROACH TO POPULATION DIVERGENCE IN CICAĐAS (HOMOPTERA, AUCHENORRHYNCHA): SOME INTRODUCTORY RESULTS\*

J.A. Quartau<sup>1</sup>, M. M. Coble, A. M. Viegas-Crespo<sup>1</sup>, M. T. Rebelo<sup>1</sup>, M. Ribeiro<sup>1</sup>, G. A. Pinto<sup>1</sup>, P. Simoes<sup>1</sup>, G. André<sup>1</sup>, M. Claridge<sup>2</sup>, J. Hemingway<sup>2</sup>, J. C. Morgan<sup>3</sup>, and S. Drosopoulos<sup>3</sup>

<sup>1</sup>Departamento de Zoologia e Antropologia and Centro de Biologia Ambiental, Faculdade de Ciências, Bloco C2, Campo Grande, 1700 Lisboa, Portugal

<sup>2</sup>School of Pure and Applied Biology, University of Wales, PO Box 915, Cardiff, UK

<sup>3</sup>Department of Agricultural Biology and Biotechnology, Agricultural University of Athens, Iera Odos 75, Athens, Greece.

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The cicadas constitute a superfamily (Cicadoidea) of the Homoptera Auchenorrhyncha, mainly distinguished by the ability of males to produce loud and distinctive airborne acoustic signals during pair formation and courtship by means of a tymbal mechanism. These signals are generally species specific and females are only attracted to the calls of the conspecific males (eg., Claridge, 1985, 1990; Quartau, 1995). Instances are common where species have suffered specific divergence without showing clear-cut morphological differences. In such cases, as within the genera *Cicada* L., and *Tettigella* Kolénati, these acoustic signals are crucial for specific discrimination (eg., Quartau and Boulard, 1995), since within each group species can be very similar in external morphology and yet the males calls may differ greatly.

The present study is centred on a pair of such closely related species within genus *Cicada* - *C. orni* L. and *C. barbara* Stål - with a view to investigate patterns of evolutionary divergence and speciation through three different approaches: behavioural, molecular and morphometric.

### 1. BEHAVIOUR

Acoustic mate recognition signals of several males of both species have been recorded in several sites in Portugal by the use of directional microphones and professional tape recorders. Male calls were analysed by computer assisted programs and oscillograms have been obtained. They proved to differ greatly between these species both in cases of allopatry and sympatry. In *C. orni* these signals consisted of a repetitive series of separate sound elements, each one in turn consisting of series of rapid and regularly reproduced pulses (eg., Quartau and Rebelo, 1994). In contrast, *C. barbara* has a continuous song, remembering in this respect those of *Tibicina* Amyot spp. Details of features such as pulse repetition frequency and pulse structure within each of the calls are also under investigation.

### 2. ELECTROPHORETIC ANALYSES

Protein electrophoresis has been applied to investigate genetic variation as population level. As techniques, starch electrophoresis, buffers and staining systems were followed as described by Pasteur *et al.* (1987). Homogenates were derived from the head and thorax isolately as well as from both parts combined. More than twenty different allozyme systems have been tested so far (cf. table), with three different buffer systems (Tris-citrate pH 6.7, Tris-citrate pH 8.0, and Tris-lithium-citrate borate pH 8.3).

Electrophoretic data from the head combined with the thorax gave better defined stains than when using these body parts separately. Moreover, the following enzymes proved to separate this pair of cicadas, malic enzyme (ME), malate dehydrogenase (MDH) and aspartate aminotransferase (AAT). As expected no differences were found between sexes within each species.

### 3. MORPHOMETRIC ANALYSES

A first set of morphometric variables has been collected to describe the body and the male genitalic morphology. Measurements were taken to describe shape and size at the level of the wings, head and abdomen as well as the aedeagus and such results are to be compared with variation at the ethological and genetic levels.

The present electrophoretic and first morphometric results confirm the ethological data, giving all strong evidence that this is a pair of two independent biological species. Larger samples of cicadas are being collected, the calls recorded, their enzymatic systems analysed and some DNA analyses are to be started in order to get a more thoroughly understanding on the population divergence of these species.



Table: Names, abbreviations, EC numbers and buffers found to be most effective in resolving enzymes from *Cicada orni* and *C. barbara*.

EC Number	Enzyme	Abbreviation	Buffer System
3.5.4.4	Adenosine deaminase	ADA	Tris-citrate pH 6.7
2.7.4.3	Adenlate kinase	AK	Tris-citrate pH 8.0
1.1.1.1	Alcohol dehydrogenase	ADH	Tris-citrate pH 6.7
1.2.3.1	Aldehyde oxidase	AO	Tris-lithium-citrate-borate pH 8.3
2.6.1.1	Aspartate aminotransferase <sup>(2)</sup>	AAT	Tris-citrate pH 6.7
2.7.3.2	Creatine kinase	CK	Tris-citrate pH 8.0
3.1.1.1/2	Esterase	Est	no staining obtained
1.1.1.49	Glucose-6-phosphate dehydrogenase	G6PDH	no staining obtained
1.1.1.47	NAD-glucose dehydrogenase	GLC	Tris-citrate pH 6.7
1.4.1.2	Glutamate dehydrogenase	GLD	no staining obtained
1.1.1.8	Glycerol-3-phosphate dehydrogenase	GPD	Tris-lithium-citrate-borate pH 8.3
5.3.1.9	Glucosephosphate isomerase	GPI	Tris-lithium-citrate-borate pH 8.3
2.7.1.1	Hexokinase	HK	Tris-citrate pH 6.7
1.1.1.42	Isocitrate dehydrogenase	IDH	no staining obtained
1.1.1.27	Lactate dehydrogenase	LDH	no staining obtained
1.1.1.37	Malate dehydrogenase <sup>(2)</sup>	MDH	Tris-citrate pH 6.7
1.1.1.40	Malic enzyme <sup>(2)</sup>	ME	Tris-citrate pH 6.7
5.3.1.8	Manosephosphate isomerase	MPI	Tris-citrate pH 8.0
	Peptidase	PEP	Tris-lithium-citrate-borate pH 8.3
5.4.2.2	Phosphoglucomutase	PGM	Tris-citrate pH 6.7
1.1.1.43	Phosphogluconate dehydrogenase	PGD	Tris-citrate pH 8.0
1.1.1.14	Sorbitol dehydrogenase	SDH	Tris-citrate pH 8.0
1.15.1.1	Superoxide dismutase	SOD	no staining obtained
1.1.1.204	Xanthine dehydrogenase	XDH	no staining obtained

<sup>(1)</sup> Best results of all three buffer systems experimented.

<sup>(2)</sup> Discriminating enzyme systems for this pair of cicadas

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