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ENZYMATIC POLYMORPHISM IN CHRYSOPERLA CARNEA (STEPHENS) AND C. KOLTHOFFI (NAVÁS) (NEUROPTERA : CHRYSOPIDAE)

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Isozymes can be used in order to study the importance of reproductive barriers between populations or species. The aim of this work is to study allozyme polymorphism in natural populations of *Chrysoperla carnea* (STEPHENS) and *Chrysoperla kolthoffi* (NAVÁS) collected in the West of France. Two enzymatic systems were studied by starch gel electrophoresis: diaphorase (DIA) and isocitrate dehydrogenase (IDH). For each enzymatic system, presumed loci were scored and genetic interpretation was proposed. The study of allozyme polymorphism at the DIA and IDH-2 loci provided evidence of marked genetic differences between *Ch. carnea* and *Ch. kolthoffi*.

Key words: lacewing, *Chrysoperla carnea, C. kolthoffi*, isozymes, electrophoresis

INTRODUCTION

The common green lacewing *Chrysoperla carnea* (STEPHENS) *sensu lato* is a cosmopolitan and eurybiote chrysopid (ASPÖCK *et al*. 1980), which has long been considered as a single cosmopolitan species, although showing local biotypes differing in seasonal and life history characters (SHELDON & MACLEOD 1974, ALROUECHDI & CANARD 1979). This situation makes the status of this species imprecise (BROOKS 1994, ASPÖCK & HÖLZEL 1996). Recent works have shown that this presumed species correspond in reality to a "complex" of morphologically and biologically different taxa (LERAUT 1991, THIERRY *et al*. 1992, 1994, 1998, DUELLI *et al*. 1996, HENRY *et al*. 1996). At least three species are recorded in the western part of Europe: *Ch. lucasina* (LACROIX), *Ch. carnea sensu stricto* and *Ch. kolthoffi* (NAVÁS) (THIERRY *et al*. 1996).

Working on determination of genetic variations by mean of electrophoresis, BULLINI and CIANCHI (1984) and CIANCHI and BULLINI (1992) in Europe and MARTINEZ WELLS (1994) in North America found some evidence supporting the existence of this complex. THIERRY *et al.* (1997) in an electrophoretic study on EST (esterase) and ACP (acid phosphatase) showed significant divergence between *Ch. lucasina* (LACROIX) and the two other common green lacewings present

in West Europe: *Ch. carnea* s. s. and *Ch. kolthoffi* (NAVÁS). The aim of this work is to study allozyme polymorphism in natural populations of this two last species.

MATERIAL AND METHODS

Collection site and identification

Sampling was carried out during the last week of August 1999, in the mid Loire valley, near the town of Angers (France), lat. 47°28'N, long. 0°33'E. The sampling zone is an alluvial area between the Loire and one of its tributaries, the Maine. The vegetation is an Atlantic type of bocage (CORILLION 1966), the arborescent vegetation being mainly composed of elm, ash and oak. At the beginning of September we can presume that a majority of adults had entered diapause (HONEK & HODEK 1976) and offered good conditions for electrophoresis (THIERRY unpublished data).

A total number of 75 adults of chrysopids were collected by hand net in the lower canopy (< 4 m) of isolated deciduous trees. After the capture, each lacewing was identified according to the criteria and the typology proposed by THIERRY *et al.* (1998) following the nomenclature proposed by LERAUT (1991) (see voucher specimens deposited in the collection of Université Catholique de l'Ouest, IRFA, F–49000, France).

The chrysopid specimens were homogenized in Eppendorf tubes containing 100 µl of extracting buffer* per specimen. Homogenates were centrifuged at 20000 g for 10 min. The supernatant was absorbed into several pieces of Whatman chromatographic paper, used as inserts in 12% starch gels. The gels were prepared according to CARDY and BEVERSDORF (1984) using hydrolyzed starch and a histidine-citrate buffer system (pH 6.5) and electrophoresed at 4°C.

Two enzyme systems were tested by appropriate staining (ACQUAAH, 1992): diaphorase (DIA) and isocitrate dehydrogenase (IDH).

Allozyme frequencies for each sample were derived from the electrophoretic results. Hardy-Weinberg equilibrium was tested using an exact test procedure. P-value of the test was calculated by means of a simulation algorithm according to GUO and THOMPSON (1992). Analyses were performed using the software package GENEPOP (RAYMOND & ROUSSET 1995).

RESULTS

Two IDH presumed loci were detected, but only the anodical one (IDH-2) was studied, the region IDH-1 not being clearly resolved. IDH-2 was found to be polymorphic for the two forms (Fig. 1).

DIA profiles were composed by several bands (Fig. 2), but the only detected variation concerned the presence or absence of the most anodical band. When present, this band was faint in some specimens but not in others. We considered this situation as the expression of a locus with two alleles, one of them being a null one, the heterozygous genotypes being recognized by the faint band.

* tris 0.1 M, polyvinylpyrrolidone 40 (8%), adjusted at pH 7.5 with HCl

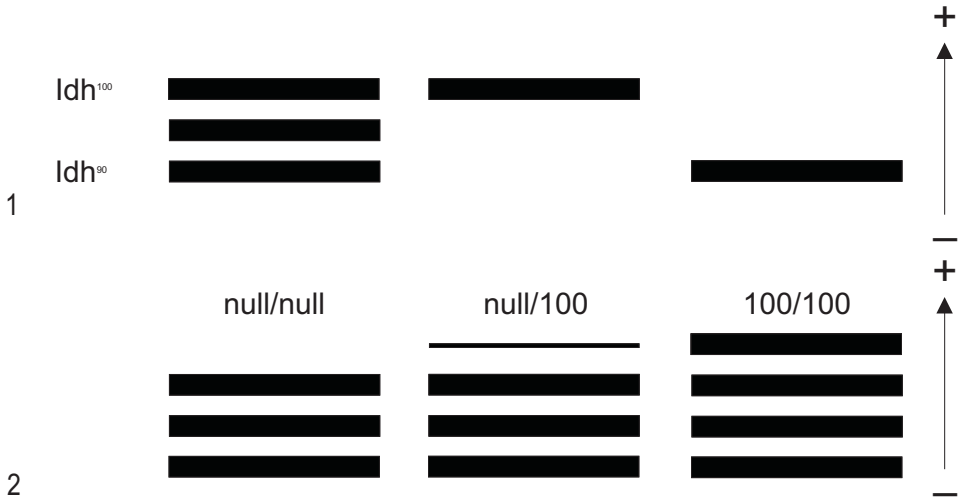
Table 1. Allele frequencies at the loci IDH-2 and DIA in *Chrisoperla carnea* (N=44) and *Ch. kolthoffi* (N = 31) and exact test for deviation from Hardy-Weinberg equilibrium

	Ch. carnea s. s.	*Ch. kolthoffi*
IDH–2^{100}	0.920	0.419
IDH–2^{90}	0.080	0.581
P-value	0.013	1
DIA^{nul}	0.917	0.556
DIA^{100}	0.083	0.444
P-value	0.126	0.698

The differences in allele frequencies between the two samples were highly significant (Table 1). Both samples were in Hardy-Weinberg equilibrium for DIA but only the *Ch. kolthoffi* sample was in Hardy-Weinberg equilibrium for IDH-2.

DISCUSSION

A low heterozygous frequency for IDH-2 in *Ch. carnea* is associated with the lack of Hardy-Weinberg equilibrium and could be attributed to a certain degree of consanguinity, but the results obtained for DIA are not in accordance with this sup-



Figs 1–2. Isozyme patterns in *Chrysoperla carnea* and *Ch. kolthoffi*: 1 = isocitrate dehydrogenase, 2 = diaphorase

position. Other factors (selection, migration preceding entering in hibernation sites) could explain the lack of equilibrium for IDH-2 but we have no evidence of this. The study of allozyme polymorphism at the DIA and IDH-2 loci give evidence of marked genetic differences between *Ch. carnea* and *Ch. kolthoffi.* These results complete those obtained by THIERRY *et al*. (1997) in their study of esterases and acid-phosphatases and are in accordance with the morphological and ecophysiological differences observed between *Ch. carnea* and *Ch. kolthoffi* (THIERRY *et al.* 1994). Ecophysiological differences in life-history (TAUBER & TAUBER 1985) and pre-mating barriers like variations in courtship song patterns (HENRY 1985) might contribute to limit gene flow between these two species.

Even if the species studied showed significantly differentiated allele frequencies, no alternative allozymes were found which could demonstrate that gene flow does not occur between those two sibling species as it has been observed in other arthropods complexes (BULLINI 1982).

*

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