SHORT NOTE

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Allozyme polymorphism in a natural population of *Chrysoperla carnea* sensu lato (Neuroptera: Chrysopidae): A contribution to the status of the constitutive taxons in western Europe

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Abstract. In recent years, several sibling species have been discovered in chrysopid lacewings. In Europe, the common green lacewing *Chrysoperla carnea* (Stephens) sensu lato seems to correspond to a "complex" of three morphologically and biologically different taxons: *Ch. carnea* sensu stricto, *Ch. kolthoffi* (Navás) and *Ch. lucasina* (Lacroix), the status of which is not yet fully clarified. We examined, by means of polyacrylamide gel electrophoresis, the esterase and acid phosphatase polymorphism in a natural population of the *Chrysoperla* complex collected in the centre of France. Five groups of esterases and two groups of phosphatases were investigated, but only two loci, Est-3 and Acp-2, achieved a good resolution allowing a satisfying genetic interpretation. The genetic analysis confirmed the significant divergence between *Ch. lucasina*, and the two others forms. The genetic similarity between *Ch. carnea* and *Ch. kolthoffi* seems to be higher. No alternative allozymes demonstrate that gene flow does not occur in field populations, nor a full reproductive isolation of the different taxons.

Introduction

The common green lacewing *Chrysoperla carnea* (Stephens) sensu lato is a cosmopolitan chrysopid, present in the whole holartic area (Aspöck et al., 1980). The ubiquitous larva is a well-known predator of small terrestrial arthropods with a wide host and prey range. Particularly, it is aphidophagous and oophagous. For these reasons, *Ch. carnea* sensu lato has long been used as an auxiliary in biological or integrated programs (e.g. Principi, 1984; Pree et al., 1989) and is the most intensively studied chrysopid (Review in Canard et al., 1984).

Chrysoperla carnea has long been considered as a single cosmopolitan species, morphologically slightly variable, although showing local biotypes differing in seasonal and life history character (Sheldon & MacLeod, 1974; Alrouechdi & Canard, 1979). But recent works show that in Europe (Henry, 1985; Lerault, 1991; Cianchi & Bullini, 1992) as in North America (Tauber & Tauber, 1985; Henry et al., 1993) different populations constitute what is more realistically called the "*carnea* complex", the status of which is not yet fully clarified (Duelli et al., 1996) and unanimously accepted (Brooks, 1994). These lacewings may differ in characteristics such as diapause physiology and choice of habitat (Thierry et al., 1994). Therefore, it is particularly important to take these facts into consideration for the utilisation of *Ch. carnea* as a biocontrol agent.

In France, Thierry et al. (1992), have used morphological criteria to distinguish three sibling species: *Ch. carnea* (Stephens) sensu stricto, *Ch. kolthoffi* (Navás) and *Ch. lucasina* (Lacroix). But they wonder about the status of non-typical individuals found in low proportion in natural populations and morphologically similar to those obtained in progeny of lab-cross between the species.

The study of gene-enzyme systems by means of electrophoretic techniques has been providing data of critical importance for taxonomists and ecologists (Avise, 1975). In this paper, we will study the electrophoretical polymorphism of acid phosphatases (EC-1.1.1.1.), esterases (EC-3.1.1.1) and malate dehydrogenases (EC-1.1.1.43), three enzymatic families widely used in taxonomy (Pintureau, 1987; Martínez Wells, 1994), as in population genetics (Roychoudhury & Nei, 1988; Parkash et al., 1993), to compare the three above mentioned sibling species of the "*carnea* complex".

Material and Methods

Collection site and identification

Sampling was carried out during the first week of September 1993, in the mid-Loire valley, near the town of Angers (France), lat. 47° 28′ N, long. 0° 33′ E. The sampling zone was an alluvian area at the confluence between the Loire and one of its tributaries: the Maine. The vegetation was an Atlantic type woodlands (Corillion, 1966), mainly composed of elm, ash and oak. Thierry et al. (1996) have showed the ability of the sampling zone to harbour the three species. *Ch. kolthoffi* prevailed in the Atlantic side of Europe, the ubiquist *Ch. carnea* appeared at a low frequency and *Ch. lucasina*, which has a southern distribution in France (Leraut, 1991), was rare. At the beginning of September the majority of adults had entered diapause (Honěk & Hodek, 1973) and offered good conditions for electrophoresis (our unpublished data).

A total number of 146 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. (1992), without considering colour variations of bristles of the costal edge, as this character has proved to be difficult to use in routine identification within our protocol. For the electrophoretic study, 105 lacewings were used: 62 adults *Ch. kolthoffi*, 26 *Ch. carnea* and 17 *Ch. lucasina*. 39 adults *Ch. kolthoffi* were excluded.

Electrophoresis

The individuals were prepared for electrophoresis by homogenizing them in toto with a teflon rod in a grinding buffer (10 mM Tris-HCl, 1 mM EDTA, 10% glycerol, 1% bromophenol blue, pH 6.8). Preparations were centrifuged for 5 minutes at 20,000 g and the clear supernatant was collected.

The gel system was a development of Davis' (1964) technics. The system includes a spacer gel 6.5% acrylamide incorporating 375 mM Tris-HCl, pH 8.8 buffer and a stacking gel 4%, incorporating 125 mM Tris-HCl, pH 6.8 buffer. The tank electrode buffer was 193 mM Tris, 25 mM Glycine, pH 8.2.

Routinely after electrophoresis, the gel was stained for esterase activity using α-naphthyl-acetate and β-naphthyl-acetate as the substrates and fast blue RR salt as diazocoupler. 35 mg of each substrate were dissolved in 1 ml acetone and this solution was mixed with 100 ml phosphate buffer pH 7 containing 50 mg of fast blue. The gel was incubated for 20 min in this solution, in the dark, with slow shaking. For acid phosphatases and malate deshydrogenases, staining recipes were taken from Pasteur et al. (1987).

Analysis

Allozyme frequencies for each sample were derived from the electrophoretic results. A factor analysis on the frequencies was performed. Hardy-Weinberg equilibrium was tested using an exact test procedure (Levene, 1949). P value of the test was calculated by means of a simulation algorithm according to Guo & Thompson (1992). Significance of genetic differentiation among taxons was tested by means of an exact test (Raymond & Rousset, in press). Analyses were performed using the software package Genepop (Raymond & Rousset, 1995). Pairwise unbiased genetic distances were calculated according to Nei (1978). The relationships among taxons were summarized in the form of a dendrogram derived from the Upgma clustering method (Sneath & Sokal, 1973).

Results

Malate dehydrogenase loci (Mdh-1 and Mdh-2) were found to be monomorphic for the three forms. Five loci of esterases were detected, but only one, Est-3 achieved a good resolution which allowed a genetic analysis of the pattern. Five alleles were recorded, respectively encoded by Est-3^{90}, Est-3^{95}, Est-3^{100}, Est-3^{105} and Est-3^{109}. Acid phosphatases showed two polymorphic loci, Acp-1 and Acp-2, but only Acp-2 allowed genetic interpretation. Four alleles were recorded, respectively encoded by Acp-2^{100}, Acp-2^{105}, Acp-2^{107} and Acp-2^{110}.

Table 1 shows the allele frequencies within the three forms. The F1 x F2 map of the factor analysis performed on this table (Fig. 1) illustrates the relative positions of the three forms and their connections with the different alleles. It particularly brings out the opposition between *Ch. lucasina* and the two other taxons.

TABLE 1. Allele frequencies at loci Est-3 and Acp-2 in the 3 taxons of the complex: *carnea*, *kolthoffi* and *lucasina* (N – sample size).

Enzymes	Forms		
	carnea	*kolthoffi*	*lucasina*
Est-3			
90	0.019	0.073	0.324
95	0.096	0.129	0.265
100	0.327	0.242	0.265
105	0.154	0.274	0.029
109	0.404	0.282	0.118
Acp-2			
100	0.435	0.565	0.000
105	0.326	0.283	0.600
107	0.196	0.152	0.400
110	0.043	0.000	0.000
N	26	62	17

All the forms are in Hardy-Weinberg equilibrium for the two loci (Table 2).

Exact tests for differentiation (Table 3) show marked differences between *Ch. lucasina* and the two other taxons. On the other hand, there are no significant differences between *Ch. carnea* and *Ch. kolthoffi*.

The Nei's standard genetic distances corrected for sample size between the different taxons are listed in Table 4. The similarities (Nei's unbiased genetic identity) between forms are summarized in Fig. 2. The clustering levels are respectively 0.998 between *carnea* and *kolthoffi*, 0.637 between *lucasina* and the two other forms. This indicates clearly the distinction of the *lucasina* taxon.

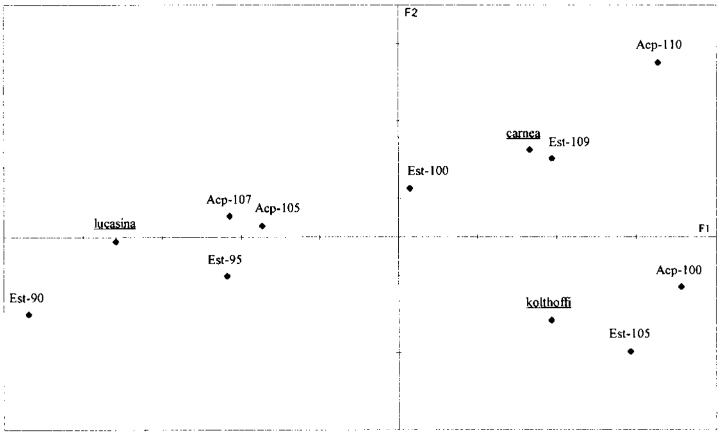

Fig. 1. F1 x F2 map of factor analysis on allele frequencies at loci Est-3 and Acp-2 in the 3 taxons of the complex: *carnea*, *kolthoffi* and *lucasina*.

TABLE 2. Exact test for deviation from Hardy-Weinberg equilibrium at loci Est-3 and Acp-2 in the 3 taxons of the complex: *carnea*, *kolthoffi* and *lucasina*.

	P-value	
	Est-3	Acp-2
carnea	0.856	0.265
kolthoffi	0.189	0.076
lucasina	0.963	0.639

TABLE 3. Exact test for pairwise differentiation at loci Est-3 and Acp-2 in the 3 taxons of the complex: *carnea*, *kolthoffi* and *lucasina*.

	P-value	
	Est-3	Acp-2
carnea x *kolthoffi*	0.154	0.438
carnea x *lucasina*	$< 10^{-4}$	$< 10^{-4}$
kolthoffi x *lucasina*	$< 10^{-4}$	$< 10^{-4}$
3 forms	$< 10^{-4}$	$< 10^{-4}$

TABLE 4. Pairwise Nei's unbiased genetic distances between the 3 taxons of the *carnea* complex.

Forms	*carnea*	*kolthoffi*	*lucasina*
carnea	–		
kolthoffi	0.001	–	
lucasina	0.417	0.483	–

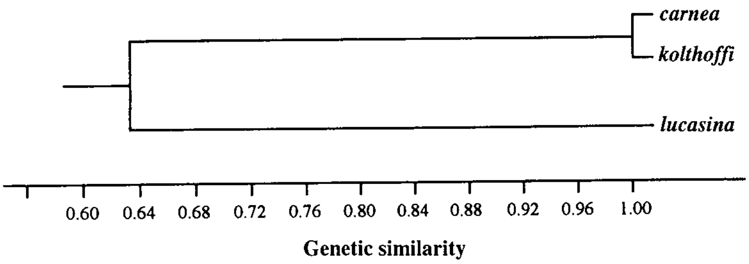

Fig. 2. Dendrogram (UPGMA method) of Nei's unbiased genetic identity among the 3 taxons of the *carnea* complex.

Discussion

The genetic analysis clearly discriminated *Ch. lucasina* from the two other populations. The genetic similarity between *Ch. carnea* and *Ch. kolthoffi* seemed to be higher. These results support the morphological and the ecophysiological inferences. The southern *Ch. lucasina* is rare in the sampling area (Lerault, 1991; our unpublished data) and shows more distinct ecophysiological differences than those observed between *Ch. carnea* and *Ch. kolthoffi* (Thierry et al., 1994). In spring, the *carnea* and *kolthoffi* forms coexist for some weeks in rolled dry leaves and in ivy tufts. This brief coexistence in the same habitat during a period of reproductive activity (Thierry et al., 1994) may allow genetic exchanges between the two populations. However, pre-mating barriers like differences in courtship song patterns (Henry, 1985) might contribute to limit this gene flow.

We find significantly differentiated allele frequencies but no alternative allozymes which could demonstrate that gene flow does not occur between *lucasina* and the two others. This study carried out with two significant enzymatic loci would not allow us to determine the status of the morphologically non-typical individuals.

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