DISTRIBUTION OF THE *CHYSOPERLA CARNEA* COMPLEX IN SOUTHERN SPAIN (NEUROPTERA: CHRYSOPIDAE)

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Abstract
Common green lacewings are major candidates for use in IPM programs because they are distributed worldwide, have a wide host plant and prey range, can be easily mass cultured, manipulated using food sprays and overwintering boxes, and pesticide tolerant populations have been identified. Although a lot of work has been carried out on Chrysopidae, but regarding the many gaps in their natural history, green lacewings are little known insects, and even their taxonomic status – at least that of the most important taxon Chysoperla carnea (Stephens, 1836) – is uncertain. It is instead of a polymorphic single species, a complex of cryptic species, the Chrysoperla carnea complex or carnea-group. In present contribution composition of the natural Ch. carnea population was investigated in order to establish systematic bases for biological control studies in olive groves of Spain. Our results based on 1158 adult lacewings, represent the biggest number of Ch. carnea complex specimens ever identified in Spain. Ch. agilis predominated with its 72.1% value. It was followed by Ch. carnea s.str. (11.6%), Ch. lucasina (5%), Ch affinis (1.4%). Regarding the number of captured specimens, it seems that Ch. agilis is the dominant species whose impact on olive moth caterpillars the greatest can be. The abundance and frequency of Ch. affinis was the smallest, and the other sibling species with their 5-12% frequency can have only more modest role in biological control of P. oleae.

Key words: *Chrysoperla carnea*, sibling species, species composition, Neuroptera, Spain

INTRODUCTION

Chrysopids have long attracted the attention of the applied entomologist for they are good candidates for use in IPM programs. They are distributed worldwide, have a wide host plant and prey range (Principi and Canard, 1984), can be easily mass cultured (Ridgway *et al.* 1980), manipulated using food sprays (Hagen and Tassen, 1980) and overwintering boxes (McEwen *et al.* 2000), and pesticide tolerant populations have been identified (Grafton-Cardwell and Hoy, 1985). Due to these characteristics in response to a survey in 1992, members of the Association of Applied Insect Ecologists ranked *Chrysoperla* ssp., the most important lacewings as unrivaled on the list of commonly used, commercially obtainable predaceous natural enemies (Tauber *et al.* 2000). Although a lot of work has been carried out on Chrysopidae, but regarding the many gaps in their natural history green lacewings are little known insects, and even their taxonomic status – at least that of the most important taxon *Chysoperla carnea* (Stephens, 1836) – is uncertain.

The systematic status of *Ch. carnea* has been changing, and instead of a polymorphic single species, a complex of sibling or cryptic species, the *Chrysoperla carnea* complex or *carnea*-group (Thierry *et al.* 1992; Thierry *et al.* 1998; Henry *et al.* 2001) should be now considered whose members` systematic status is not known enough (Tauber *et al.* 2000; Henry *et al.* 2001; Canard and Thierry, 2005). Several attempts of multiple approaches such as courtship sonification (Henry, 1983, 1985), genetic studies with multilocus electrophoresis (Cianchi and Bullini, 1992), nucleotide sequences of COII, cytochrome oxidase I, cytochrome b and the large ribosomal subunit of the mtDNA (Lourenço *et al.*

2006), morphological characterization of adults and larvae (Thierry *et al.* 1992), ecophysiological variability (Thierry *et al.* 1994; Canard *et al.* 2002) have been made.

They supported the existence of various cryptic species among which one can find: 1) *Ch. carnea* former *Chrysoperla kolthoffi* (Navás, 1927) sensu Cloupeau (*Cc4* as song species), or "motorboat"(as song type) (Henry *et al.* 2002) or *Chrysoperla affinis* (Stephens, 1836) former *Ch. kolthoffi* (Thierry *et al.* 1998); 2) *Chrysoperla lucasina* (Lacroix, 1912) (Henry *et al.* 2001) and 3) *Chrysoperla carnea* (Stephens, 1836) sensu stricto (Thierry *et al.* 1998) or *Cc2* ("slow-motorboat") or *Chrysoperla pallida* sp. nov. (Henry *et al.* 2002); 4) *Chrysoperla agilis* Henry, Brooks, Duelli and Johnson 2003 (Henry *et al.* 2003) or *Cc3* (Maltese).

In spite of the efforts made for clearing the taxonomic status of Palearctic *Ch. carnea* the present situation of species demarcations cannot be called satisfying because of the lack of agreement in reliable criteria (Tauber *et al.* 2000; Henry *et al.* 2001, 2002; Canard *et al.* 2002; Canard and Thierry, 2007). There is a deep controversy between the two main groups of researchers (one of them uses mainly the substrate-born vibrational songs and certain morphological characteristics like shape of the male genital "lip" and "chin", another prefers ecophysiological traits and subtle morphological differences (like shape of the basal dilatation of the metatarsal claw, pigment distribution of the stipes, etc.) for distinguishing the cryptic species of *carnea*-group) because the first group concluded that the true *Ch. carnea* described by Stephens in 1836 and to be found in The Natural History Museum, London, must be *Cc4* (Henry *et al.* 2002) which according to the other group is another species, the *Ch. affinis* (Canard and Thierry, 2007). The other candidate species for being the "true" *Ch. carnea* may be *Cc2* mentioned above like *Ch. carnea* s. str. (Canard and Thierry, 2007) but in contrast with it, this taxon was assigned a new name, *Chrysoperla pallida* sp. nov. by Henry *et al.* (2002). Regarding the lack of perfect evidences and the somehow too complicated argumentation about the consideration of the decisive traits, the validity of these names, however, has not been discussed and accepted by the neuropterist community yet (Canard and Thierry, 2007).

These are the theoretical or taxonomic troubles. But which are the practical ones? It should not forget the natural enemy role of *Ch. carnea*, because this taxon has been used in green houses and in the fields and orchards. It has been reared, tested, qualified and sold worldwide. It is a species about which a great deal of articles have been written. Main questions: Which taxon was the object of these studies? Which taxon can we buy at Koppert or Biobest? Which taxon's natural populations help growers in various countries? Regarding the study of the carnea group, there are considerable gaps almost everywhere: in Europe, America, Asia, Australia and Africa. We know only very little about the presence, distribution, ecological demands, preferences, habitats of the taxon which formerly was called as *Chrysoperla carnea*. However, there are some countries where, due to the work of few neuropterists, the natural common green lacewing populations have somehow been characterized. These countries are France, the USA, Switzerland, England, Germany, Belgium, Romania and Hungary. Reducing our examination only to the European continent, it is a fact that the not mentioned European countries' common green lacewings represents white spots on the map of our knowledge. The following study tries to take the first steps regarding the *Ch. carnea* complex research in Spain.

Olive moth (*Prays oleae* (Bernard, 1788)(Lepidoptera: Plutellidae) is one of the most important insect pests of olive groves in the Mediterranean basin and so is in Andaluzia (Spain is the biggest olive oil producer in Europe) as well. The second generation females lay eggs on the small fruits in early summer, and the emerging larvae bore within the olive fruit causing spectacular fruit drop in July and August (Ramos *et al.* 2005). Various methods are used against the moth population but in most cases insecticides are applied (Ramos *et al.* 2005). Taking into consideration the environmental and human feeding risks the development of integrated or biological control methods would be necessary for the environmentally friendly or organic production of olives. According to local observations the common green lacewing (*Chrysoperla carnea* (Stephens, 1836) sensu lato) may be an efficient predator of the olive moth eggs and caterpillars (Al-Asaad, 2004). However, which sibling species is the really efficient taxon?

In some years when the density of lacewings is proper, the natural control is efficient. However, in other years the density is small, and there is no natural control by lacewing larvae (Ramos *et al.* 2005). That is why the following questions can be raised: How is it possible to forecast the lacewing density? How can we improve the density of natural populations?

Possible solutions:

- identification of the lacewing species (sibling species) controlling olive moth caterpillars,
- measuring the predatory performance of lacewing larvae using *in situ* observation and laboratory experiments,
- study of population dynamics of lacewings and its dependence on major environmental factors
- determination the chrysopid fauna of some Andaluzian olive plantations and characterization of their population dynamics,
- measuring the efficiency of food sprays and over-wintering boxes for possible augmentation and conservation of common green lacewing adults,
- studying the impact of uncultivated areas for natural lacewing populations, mainly for their maintenance, over-wintering and distribution.

On the basis of these data it will be possible to develop a conservation and augmentation strategy for the natural populations as well as to select the best fitted species for the control and the probable rearing procedures. The possible utilization (a biological control technology can be developed for Spanish olive producers) of results will help to reduce the plant protection charges in olive production and diminish the environmental contaminations by pesticides in the end products. With all these activities we can considerably contribute to the production of healthier and better quality food and also to the maintaining of declining biodiversity at European level.

MATERIAL AND METHODS

Ch carnea s. l. adults were collected in 2003 and 2004 in olive groves in Jaén and Granada counties and in 2005 in the park and adjacent orchards of Granada (in the southeast of Spain). Captures were obtained by chromatic sticky traps (yellow and blue), olfactory traps, light traps and sweeping net. Individuals were identified according to the descriptions of Thierry *et al.* (1992), Henry *et al.* (2003), Duelli, (1995) and also samples of various morphological types (courtesy of Thierry, D.) and song morphs (courtesy of Duelli, P.) have been used. In case of *Ch. agilis* and *Ch. affinis* atypical specimens were excluded. The individuals captured by sticky traps and light traps were collected, put into ethanol and identified several weeks later. In case of the sweeping, living specimen were identified immediately after catching. Table 1 contains the basic data of sampling.

Table 1.

Basic data of collection in southern Spain

Site	Geographical position	Habitat	Year	Catching method	Number of individuals caught
Ubeda	43°19' N 7°22'W 551 m	olive grove	2003	coloured sticky traps	207
Torrequebradilla	37°55' N 3°40'W 397 m	olive grove	2004	coloured sticky traps	418
La Nava	38°40' N 5°24'W 664 m	olive grove	2004	coloured sticky traps olfactory traps	370
Láchar	37°12' N 3°49'W 556 m	olive grove	2004	coloured sticky traps	63
Fuerte del Rey	37°52' N 3°52'W 437 m	olive grove	2004	coloured sticky traps	24
Granada	37°15' N 3°15'W 662 m	park, mixed orchards	2005	sweep net	76

RESULTS AND DISCUSSION

Abundance values of the species caught in southern Spain are presented in Table 2. *Ch. agilis* predominates without question with its 72.1% value. It is followed by *Ch. carnea* s.str. (11.6%), *Ch. lucasina* (5.0%) and *Ch affinis* (1.4%). *Ch. carnea* s.l. (9.8%) represents the undeterminable individuals whose identification because of their morphological injury, the great quantity of unremovable glue remains on their body or the considerable variability of their characteristic traits was not possible.

Table 2.

Number and proportion of sibling species of common green lacewings collected in Andaluzia

Sites	*Ch. agilis* ind. (%)	*Ch. carnea* s. stricto ind. (%)	*Ch. lucasina* ind. (%)	*Ch. affinis* ind. (%)	*Ch. carnea* s. lato ind. (%)	Total
Ubeda 2003	125 (60.4)	44 (21.3)	25 (12.1)	9 (4.3)	4 (1.9)	207
Torrequebradilla 2004	275 (65.8)	75 (17.9)	16 (3.8)	4 (1.0)	48 (11.5)	418
La Nava 2004	287 (77.6)	14 (3.8)	14 (3.8)	1 (0.3)	54 (14.6)	370
Láchar 2004	57 (90.5)	-	1 (1.6)	-	5 (7.9)	63
Fuerte del Rey 2004	20 (83.3)	1 (4.2)	1 (4.2)	-	2 (8.3)	24
Granada 2005	71 (93.4)	1 (1.3)	1 (1.3)	2 (2.6)	1 (1 .3)	76
Total	835 (72.1)	135 (11.6)	58 (5.0)	16 (1.4)	114 (9.8)	1158

The four sibling species of the *Ch. carnea* complex have been found in the olive groves of Andaluzia. The species with the highest number of individuals collected was *Ch. agilis*. It is one of the sibling species that is the most difficult to diagnose from preserved or alive caught individuals (Henry *et al.* 2003). As to their identification on the basis of vibrational patterns, any of the European neuropterists – with the exception of a minority (mostly American) searchers developed the methodology – did not have the facilities and opportunities to verify them. It is to note, that – for the identification with vibrational pattern - one needs living and fit individuals of both sexes as well as a sophisticated appliance because the pattern shall be measured and recorded *in copula* of the lacewings (Henry, 1983). *Ch. agilis* is easily confused with the most often occurring species of the *carnea* group, *Ch. affinis* and *Ch. carnea* s.str. (Canard *et al.* 2002). In addition, there is another possibility for confusion as these dominant European lacewing taxa are named differently (see above). Besides the systematic difficulty also their distribution and occurrence have not been studied properly. The descriptors of the species analyzed 74 individuals whose origin is shown in Table 3.

Our results based on 1158 lacewings, the biggest number of *Ch. carnea* complex specimens ever identified in Spain. According to these data, those of Henry *et al.* (2003) and Canard *et al.* (2002), the occurrence of *Ch. agilis* is common in southern Spain, in the Mediterraneum and the species occurs in Central-Asia (Iran) as well. Besides *Ch. agilis*, *Ch. lucasina*, another rather Mediterranean or Atlanto-Mediterranean lacewing (Henry *et al.* 2002; Bozsik *et al.* 2003) and *Ch. carnea* s.str and *Ch. affinis*, two in the mainland Europe dominant species (Thierry *et al.* 1996; Bozsik *et al.* 2003) have been collected. Regarding the number of captured specimens, it seems that *Ch. agilis* is the dominant species whose impact on olive moth caterpillars can be the greatest. The abundance and frequency (1.4%) of *Ch. affinis* was the smallest, and the other sibling species with their 5-12% frequency can have only less significant role in biological control of *P. oleae*.

Considering the planned research activity indicated in the introduction, all the studies mentioned there should be done firstly on *Ch. agilis.*

Table 3

Collection sites and abundance of *Ch. agilis* (on the data of Henry *et al.* 2003)
(No data = the sites were indicated as collection places but the number of collected specimens was omitted)

Local site	Country	Date	Number of individuals
Azores archipelago	Portugal	August 2000	16
Southern Spain (Alicante, Granada)	Spain	July 2001	4
Southern France(Carcès)	France	August 1994	5
The Alps (Ticino)	Switzerland	1981-94	10
Southern Italy and Sicily	Italy	July 1993	21
Malta	Malta	1991	4
Xilokastron, Kalentzi and other sites	Greece	June 1994	10
Eilat	Israel	October1993-94	4
Northern Iran	Iran	June 2002	No data
Agadir	Morocco	1985	No data
Total number of individuals			74

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