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First data on the sibling species of the common green lacewings in Spain (Neuroptera: Chrysopidae)

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Summary

- Common green lacewings are good 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 *Chrysoperla carnea* (Stephens) – 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 940 lacewings, represents the biggest number of *Ch. carnea* complex specimens ever identified in Spain. *Ch. agilis* predominated with its 77% value. It was followed by *Ch. carnea* s.str. (8%), *Ch. lucasina* (6%), *Ch. affinis* (2%). 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 6-8% frequency can have only more modest role in biological control of *P. oleae*.

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The taxonomic status of the most important lacewing species in Andalusia



Introduction

- Chrysopids and among them the common green lacewings are not only attractive and wonderful insects but also good candidates for IPM programs because
- they are distributed worldwide (Principi & Canard, 1984),
- have a wide host plant and prey range (Principi & Canard, 1984),
- can be mass cultured (Ridgway et al., 1980)
- manipulated using food sprays (Hagen & Tassen, 1980),
- overwintering chambers (McEwen et al., 1999)
- pesticide tolerant populations have been identified (Grafton-Cardwell & Hoy, 1985).
- However, there is something which can made uncertain all these data. This is the present taxnomic status of *Chrysoperla carnea*.

Systematic troubles

- The original name of the species was *Chrysopa carnea*. However, because of taxonomical accuracy (nervature and genitalia) Steinmann (1964) created the genus *Chrysoperla*, and *Chrysoperla carnea* was long considered highly polymorphic, as reflected by the numbers of varieties, subspecies, e.g. 29 in Navás (1915), eight in Steinmann (1967), 16 in Aspöck et al. (1980), 14 in Brooks (1994) and 80 in Duelli (unpubl.).
- The taxonomic 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).

Attempts for clearing the taxonomic status of *Chrysoperla carnea* s.l.

Methods	References
courtship sonification	Henry, 1983, 1985
genetic studies with multilocus electrophoresis molecular systematics	Cianchi and Bullini, 1992 Henry et al., 1999
morphological characterization of adults and larvae	Thierry et al., 1992
ecophysiological variability	Thierry et al., 1994; Canard et al., 2002
AChE tolerance	Bozsik et al., 2002 unpublished

Courtship sonification (Henry, 1994; Henry et al., 2002)

- Silent inaudible sing: the male places himself in front of the female and starts to oscillate his abdomen vertically at 30-120 Hz, shaking the substrate.
- The female with her extremely sensitive mechanoreceptors in the tibiae are tuned sharply to the frequency range characteristic of the species.
- If the frequency sang by the male suits the frequency range characteristic of the species, the female answers, and they sing in duet and the copulation takes place.
- In case of singing improper frequency, the female does not accept the male.
- However, sibling species of the complex hybridize in the laboratory when given no choice. The progenies of these crossings are viable and fertile.

Weak points of courtship sonification

- nobody could verify the method except Henry and colleagues
- the methodology is too complicated, unsuitable for identifying a great number of individuals
- only living insects can be used,
- females and males should be maintained in the laboratory,
- long observation period is needed under perfect conditions for the lacewings in order to be ready for copulation,
- tremulation should be recorded
- influence of the recordings should be verified

Genetic studies with multilocus electrophoresis and molecular systematics

- Regarding multilocus electrophoresis there are differences but variation is too considerable, because Cianchi and Bullini (1992) unrecognized the sibling species
- Relationships among Eurasian species are ambiguous according to molecular systematic results (Henry et al., 1999)

Morphological characterization of adults (Thierry et al., 1992)

- colour of ventral setae on the distal portion of the abdomen: blond/light versus black/brown
- dark markings on the maxillary stipes: slight/point versus extensive/stripe
- shape of the basal dilatation of the hind pretarsal claws: broad versus narrow
- length of setae on the costal parts of fore wings: short versus long
- colour of dorsal setae on the pronotum: blond/light versus black/brown
- dark brown stripe on the pleural membrane of the second abdominal segment
- colour of genae: green versus reddish
- manifestation of overwintering coloration: green versus reddish/yellowish

Weak points:

- considerable variation in case of some characteristics

Morphological characterization of adults (Henry et al., 2002)

- fine structure of the male distal abdomen
- **Weak points:**
 - only males can be identified
 - dry or pinned material can be determined with difficulty if possible
 - the implementation of the method is too slow
 - nobody used it for identification of many lacewings

Ecological differences (Thierry et al., (1994), Henry et al., (2002)

- *Ch. carnea* is an arboreal species
- *Ch. affinis* prefers croplands and meadows
- *Ch. lucasina* prefers croplands, too

Ecophysiological variability Thierry et al. (1994)

Overwintering places

- *Ch. carnea* hibernates in rolled dry leaves and in ivy tufts
- *Ch. affinis* hibernates indoors (in buildings)
- *Ch. lucasina* overwinters in ivy tufts

Recovery of vitellogenesis

- Two week delay in recovery of reproductive activity between *Ch. carnea* and *Ch. affinis*

AChE tolerance (Bozsik et al., 2002 unpublished)

- Variation in tolerance level can be important.
- Further work is needed.

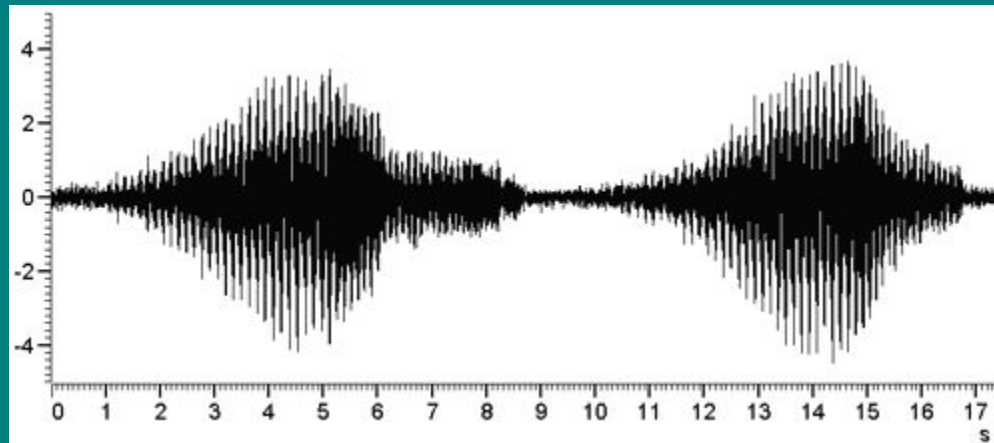
The different evidences supported the existence of various cryptic species

- *Ch. carnea* former *Chrysoperla kolthoffi* (Navás, 1927) sensu Cloupeau (Cc4 as song species), or “motorboat”(as song type) (Henry et al., 2002) or *Ch. affinis* Stephens, 1836 former *Ch. kolthoffi* (Thierry et al., 1998)
- *Chrysoperla carnea* sensu stricto (Thierry et al., 1998) or Cc2 (“slow-motorboat”) or *Chrysoperla pallida* sp. nov. (Henry et al., 2002).
- *Chrysoperla lucasina* (Lacroix, 1912) (Henry et al., 2001)
- *Chrysoperla agilis* sp. nov. (Henry et al., 2003) or Cc3 (Maltese)

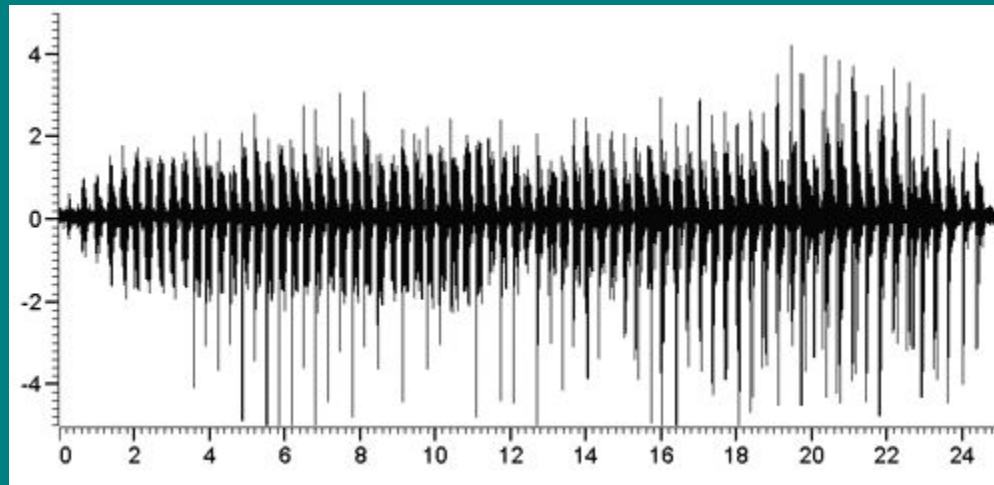
Oscillographs of some European *Chrysoperla* spp.

- Source:
- http://www.eeb.uconn.edu/people/cherry/Cryptic_songs.html
- The Cryptic Song Species of *Chrysoperla*
- Charles S. Henry, Department of Ecology & Evolutionary Biology, University of Connecticut

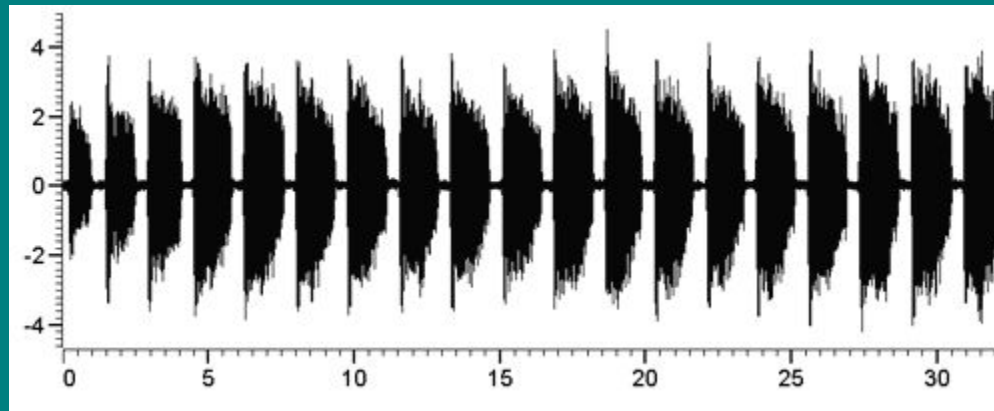
Oscillograph of *Ch. affinis* (Cc4, motorboat)



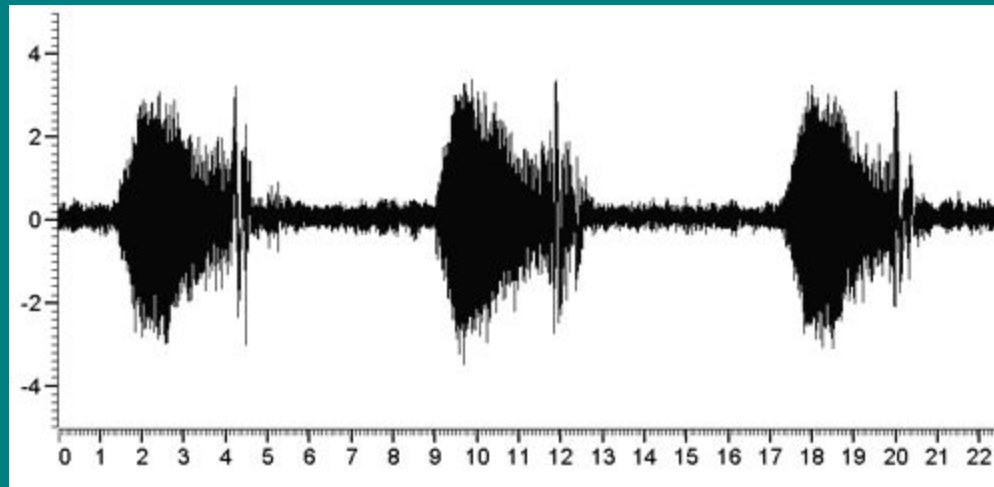
Oscillograph of *Ch. carnea* s.str. (Cc2, slow-motorboat)



Oscillograph of *Ch. lucasina*



Oscillograph of *Ch. agilis* (Cc3, Maltese)



Morphology of Chrysoperla spp.



Ch. affinis



Ch. affinis (female)



Ch. affinis



Ch. carnea s.str.



Ch. carnea s.str.



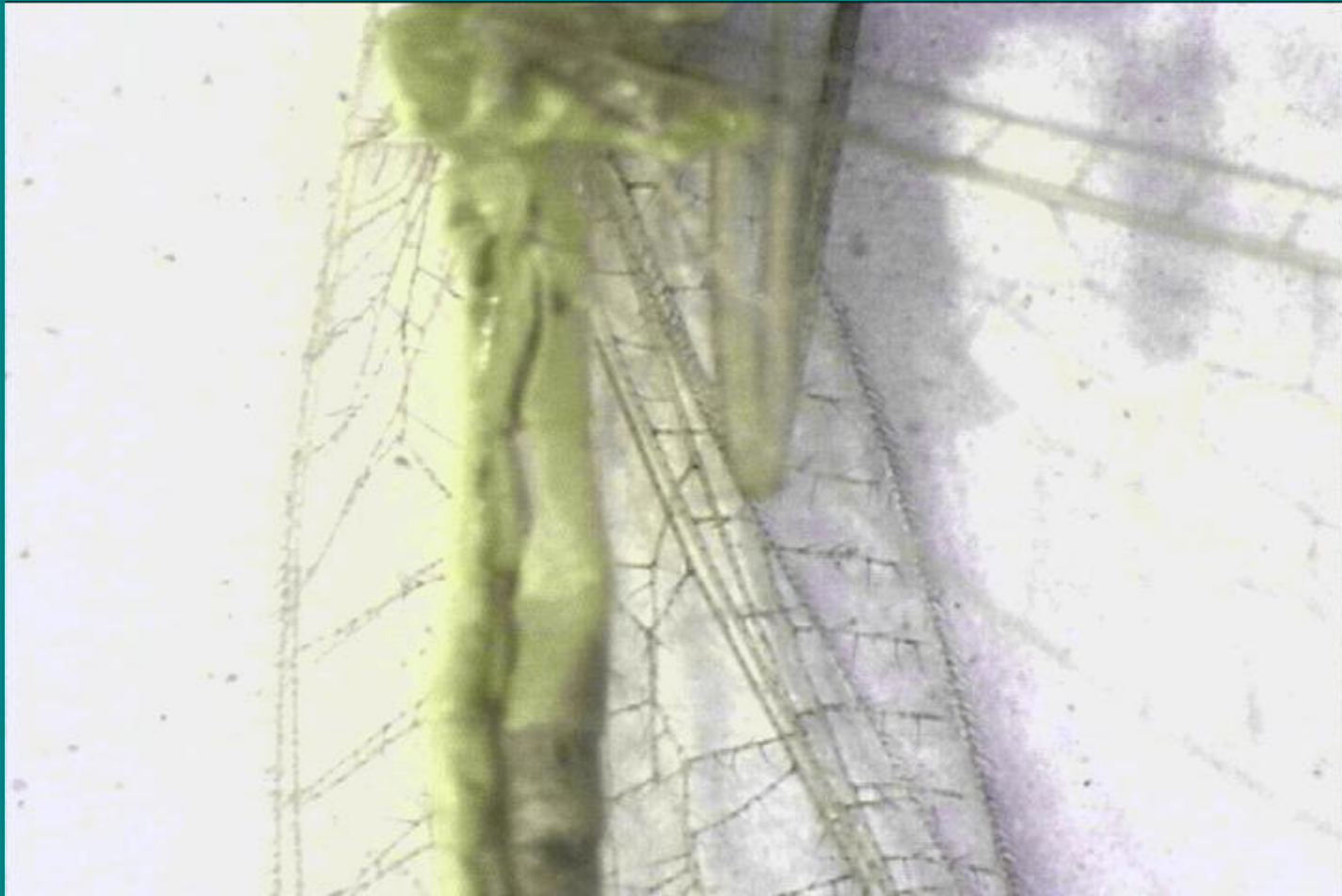
Ch. carnea s.str.



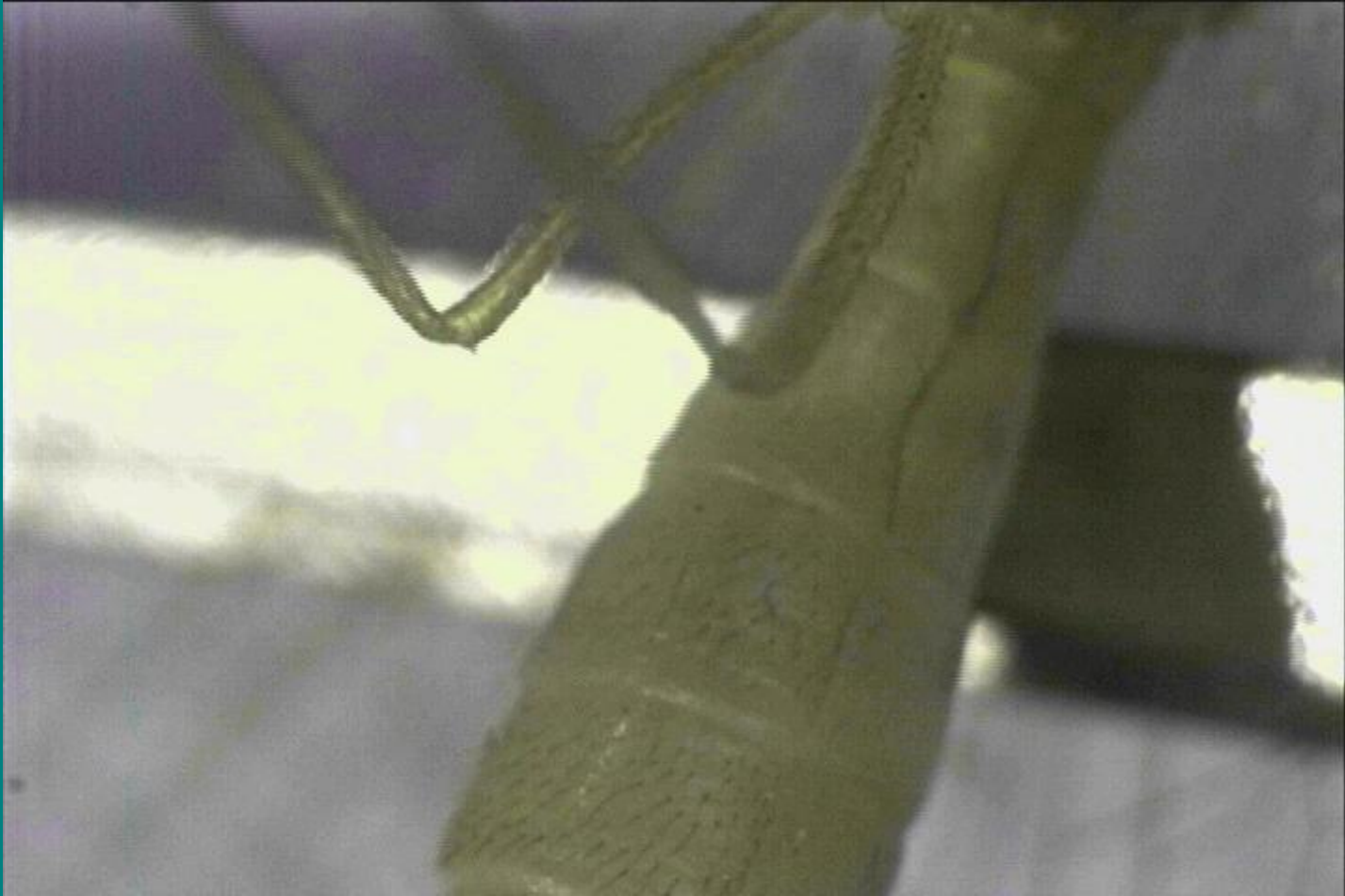
Ch. lucasina



Ch. lucasina



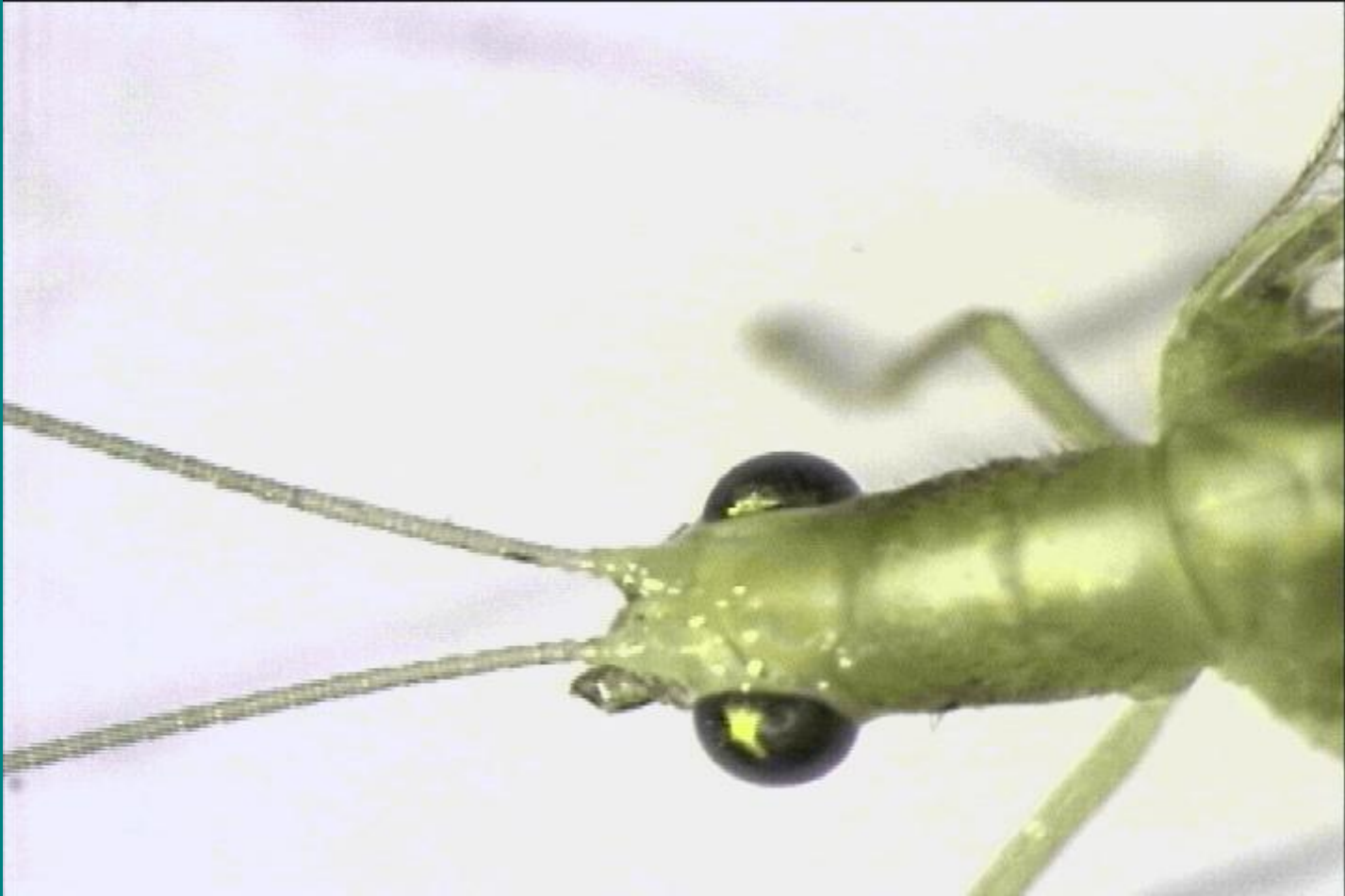
Ch. lucasina



Ch. lucasina (female)



Ch. lucasina



Practical troubles

- One should not forget the natural enemy role of *Ch. carnea*, which is used in green houses and in the fields and orchards. It is reared, tested, qualified and sold worldwide. A species about which many-many 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 helps growers in various countries?
- Nobody knows them (everybody used mixed populations)

A practical example of the study of *Ch. carnea* complex

- Management of the most important natural enemy of *Prays oleae* in Spain

Natural control for olive moth

- Olive moth (*Prays oleae* (Bernard)) is one of the most important insect pests of olive groves in the Mediterranean basin and so also in Spain, Andaluzia.
- 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).
- Control of pest: in most cases insecticides are applied (Ramos, Ramos, González, 2005). Considering 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 larvae of the common green lacewing (*Chrysoperla carnea* (Stephens) s. l.) may be an efficient predator of the olive moth eggs and caterpillars (Al-Asaad, 2004).

Questions

- 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.
- a. How is it possible to forecast the lacewing density?
- b. How can we improve the density of natural populations?

Future tasks

- a.
 - 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,
- b.
 - study of population dynamics of lacewings and its dependence on major environmental factors
 - 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, overwintering and distribution.

Basic data of collection in southern Spain

Site	Habitat	Year	Catching method	Number of individuals caught
Ubeda	olive grove	2003	coloured sticky traps	207
Mancha Real	olive grove	2004	coloured sticky traps	203
La Nava	olive grove	2004	coloured sticky traps olfactory traps	367
Láchar	olive grove	2004	coloured sticky traps	63
Fuerte del Rey	olive grove	2004	coloured sticky traps	24
Granada	park, mixed orchards	2005	sweep net	76

A typical olive grove landscape

(Photo by R. González Ruiz)



Olive trees

(Photo by R. González Ruiz)



Identification

- Individuals preserved in ethanol were identified according to the descriptions of Thierry *et al.* (1992), Canard, M. (2002 and 2003, pers comm.), Duelli, P. (1995 and 1999, pers comm.) and also samples of various morphological types (courtesy of Thierry, D.) and song morphs (courtesy of Duelli, P.) have been used. Atypical and damaged specimens were excluded.

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

Sites	<i>Ch. agilis</i> ind. (%)	<i>Ch. carnea</i> s. <i>stricto</i> ind. (%)	<i>Ch. lucasina</i> ind. (%)	<i>Ch. affinis</i> ind. (%)	<i>Ch. carnea</i> s. <i>lato</i> ind. (%)	Total
Ubeda 2003	125 (60.4)	44 (21.3)	25 (12.1)	9 (4.5)	4 (1.9)	207
Mancha Real 2004	165 (81.3)	17 (8.4)	13 (6.4)	4 (1.9)	4 (1.9)	203
La Nava 2004	287 (78.2)	11 (3.0)	14 (3.8)	1 (0.3)	54 (17.7)	367
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	725 (77.1)	74 (7.9)	55 (5.9)	16 (1.7)	70 (7.4)	940

Collection sites 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	October 1993- 94	4
Northern Iran	Iran	June 2002	No data
Agadir	Morocco	1985	No data
Total number of individuals			74

Conclusions

- *Ch. agilis* is the dominant species in Southern Spain and the olive groves as well.
- Further research should focus this species.
- *Ch. carnea* s.str. and *Ch. lucasina* can contribute to the useful performance of *Ch. agilis*.

By biologic control and IPM we can
save landscapes like that



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