

# Biogeographic origin of the common green lacewings (Neuroptera, Chrysopidae) of the Azores archipelago, through morphology analysis

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Due to a great dispersal ability and small size, insects are probably among the first island colonisers. *Chrysoperla carnea sensu lato* was for long considered the sole member of the Chrysopidae family in the Azores (Portugal) since it has been regarded as a complex of sibling species in Europe and North America. Its separation into different morphotypes is based on a set of pre-determined morphological traits. Their analysis leads to appraise Azorean Chrysopidae origin, comparing insular and mainland populations. Two outside “*carnea* complex” species, namely the Nearctic *C. rufilabris* and the Neotropical *C. externa*, were also examined to validate the method. Data analysis resulted in grouping separately the two out-group species as expected, and clearly joins insular and Palaeartic specimens of *C. lucasina*. *Chrysoperla agilis*, although known to be present in the Azores through courtship song analysis, is morphologically similar to the two other Palaeartic species, which confirms the continental Palaeartic origin of the common green lacewings in the Azores. Madeira has probably served as a stepping-stone to the Azorean colonisation.

Key words: *Chrysoperla carnea*, Macaronesia, morphotypes, Palaeartic origin

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## INTRODUCTION

The Azores are a group of nine volcanic oceanic islands located remotely in the North Atlantic Ocean. Origin and establishment of their arthropod populations are still insufficiently known. Because of their considerable dispersal ability and small size, insects are able to travel long distances and to arrive at these remote sites through different ways, such as commercial exchanges between islands and mainland (e.g. Baez 1990). Among the Macaronesian archipelago, the young geological age and

geographical isolation of the Azores (e.g. Beyhl et al. 1995) supports their less diverse and underdeveloped indigenous fauna (Evers et al. 1973). This has probably contributed to a greater susceptibility to human introductions (Ohm & Klemmer 1973). Since inhabitants originated mostly from the Iberian Peninsula, this is also the origin of most of the alien species (Le Grand 1984) so that there is a continental Palaeartic origin for the majority of indigenous insect species (Bivar de Sousa 1985). Wallace (1872) already reported the presence of 90 % of continental European *taxa* in the islands' fauna.

Due to the distance separating the archipelago from the nearest continent, the neighbouring Madeira archipelago has been pointed out as the most probable “stepping-stone” to the Azorean colonisation (Balletto et al. 1990), being closer to the continent and of older geological age. Concerning the neuropterological fauna, Ohm (1973) identified the biogeographical affinities that existed between the two archipelagos. This insect order is now recorded in all of the Macaronesian archipelagos (Aspöck et al. 2001), but with different species richness. In 1963, Tjeder recorded in the Azores the presence of *Chrysoperla carnea* (Stephens 1836), the sole member of the Chrysopidae in the archipelago. Later expeditions led to the same conclusion (Ohm 1973; Aspöck et al. 1980; Ohm & Hölzel 1982, 1984). Recently, Ventura et al. (2005) reported the presence of *Chrysoperla agilis* Henry, Brooks, Duelli & Johnson, 2003 and *Chrysoperla lucasina* (Lacroix, 1912).

Since the discovery in North America and Europe of a complex structure within *C. carnea* (Henry 1983), this species remains designated as “common green lacewing” when the constituting sibling species cannot be identified specifically. The species was referred to in the Azores in this way in the recent Neuropterida revision undertaken by Aspöck et al. (2001). In Madeira, this species shares its habitat with *Atlantochrysa atlantica* (Mclachlan 1882), an endemic species to Macaronesia reported from Madeira by Ohm & Hölzel (1984).

The recognition of the different courtship songs within the common green lacewings enables the existence of a reproductive isolation within the species complex and supports the existence of different cryptic species, based upon the biological species concept. This concept was proposed initially by Mayr (1963) who defended that a population is considered as a species if it is isolated reproductively from other sympatric populations. However, the observation that fertile hybrids may be obtained under laboratory conditions may question this conclusion or be a sign that reproductive isolation mechanisms are not yet fully operational. The rareness or the absence of hybrids in the field may point out a reproductive barrier that is indeed efficient. The only certain mechanism of fast identification

of a species by entomologists must be through morphological traits which are sometimes difficult to apply on cryptic species. The first successful approach to this problem was elaborated by Thierry et al. (1992). Nonetheless, there is a significant amount of morphological variation rendering confusion in the establishment of typical morphotypes. Moreover, since the Azorean islands are small and homogeneous ecosystems when compared to the adjacent continents, hybridisation phenomena are more likely to occur.

The present study aims primarily to verify if morphological traits in Azorean individuals are similar to those found in individuals from the West-Palaeartic mainland, confirming the results obtained by courtship song analysis (C. Henry pers. comm.), and also to verify the West-Palaeartic origin of the Azorean populations. Another goal is to establish if individuals are typical morphotypes.

## MATERIAL AND METHODS

### AZOREAN GEOGRAPHIC CHARACTERISATION

The Azorean archipelago is located in the Atlantic Ocean, about 38 °N and 28 ° W, at about 1300 km from continental Europe, and 1900 km from Newfoundland. It is constituted of three groups of islands (Fig. 1). The climate of the Azores is characterised by mild temperatures which average 17.5 °C at sea level, and a high relative humidity (Agostinho 1938a), both conditions favouring the establishment of the associated entomological fauna.

### CAPTURE OF CHRYSOPIDAE

A preliminary survey was undertaken during summer, in the most representative insular landscapes (agroecosystems, orchards and forests), on several islands of the three groups (Fig. 1). Collections never took place above the 400 m topographic contour, since previous studies have shown that no specimens were ever captured above an altitude of 250 m (Ohm 1973), an observation confirmed by the present survey. During the following summer, the sites with a higher incidence of Chrysopidae were revisited, namely cornfields and citrus orchards lying close

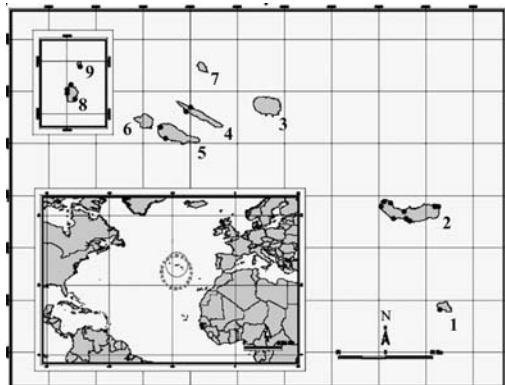


Fig. 1. Azores in the NA context; (1) Sta Maria\*, (2) S. Miguel\*, (3) Terceira, (4) S. Jorge\*, (5) Pico\*, (6) Faial, (7) Graciosa, (8) Flores\* and (9) Corvo\* island; \*sampling locations in black dots.

to sea level, to improve the sampling strategy. The sampling took place between June and August, i.e. the time between exit and enter to diapause's sites at these latitudes.

Different collecting techniques have been tested: light traps, attractive traps, hand net, visual search; finally, the more efficient approach has been the visual search in the vegetation, with a sampling effort of two people for two hours. All of the life cycle stages of the insects were captured. Adults were kept alive until identification and then preserved in a 9:1 (70 % alcohol : glycerol) solution in the dark; immature stages were reared until adulthood in individual vials, fed on eggs of *Ephestia (Anagasta) kuehniella* (Zeller, 1839).

In addition to the insular collection, specimens of common green lacewings caught in biogeographic regions of interest to the present study, were provided by local researchers (Table 1). Adults were identified according to the morphological traits defined by Thierry et al. (1992). These traits present diagnosing modalities (Table 2) used to group the specimens into different morphotypes.

#### DATA TREATMENT

In order to validate the morphotypes into species complex, a multiple correspondence analysis was undertaken; this combined qualitative and quantitative variables on the data matrix. The analysis was undertaken upon the disjunctive

table; in this each modality, of each morphological trait, assumes a value of 1 if the individual possesses the trait or 0 if it does not.

The data matrix consisted initially of 729 lines x 21 columns, with the lines representing the individuals analysed and the columns representing the variables.

Table 1. Origin, abundance and sampling sites of Chrysopidae populations used in the analysis.

Country	Locality	Abund.	Habitat
Portugal	Mainland	28	Orchards
Spain	Punta de Miro	1	?
	Peniscolla	3	?
France	Brittany	7	Corn Field
	Angers	20	<i>Frasncsinus</i> sp.
	Ang., Ile S'Aubin	34	<i>Quercus</i> sp.
	Drome	3	<i>Quercus</i> sp.
	Rochecorbon	1	<i>Quercus</i> sp.
	Fontcouvert	3	?
Kresnensko Defile (undetermined)		4	(light trap)
		18	?
Germany	Augsbourg	1	<i>Frasncsinus</i> sp.
Italy	Rome	4	Bushes
Yugoslavia	Rijeca	11	Bushes
Croatia	Fazania	4	<i>Salix</i> sp.
Greece	Kalabaka	6	?
Hungary	Györ	12	Bushes
	Zirc	6	?
Bulgaria	Kresnensko	4	<i>Quercus</i> sp.
	Sinemorec	2	?
Poland	Slupsk	8	Bushes
Canada	(undetermined)	36	Mass production
Brazil	Lavras (MG)	36	Citrus Orchard
<b>Total</b>		<b>252</b>	
Portugal, Madeira	Madeira	7	Herbac. veget.
	Madeira	7	<i>Hibiscus</i> sp.
	Madeira	9	Peach Orchard
<b>Total</b>		<b>23</b>	
Portugal, Azores	Sta. Maria	2	Citrus Orchard
	São Miguel	10	Herbac. veget.
	São Miguel	129	Corn Field
	São Miguel	5	Citrus Orchard
	Pico	5	Flower Garden
	Pico	54	Corn Field
	Pico	23	Citrus Orchard
	São Jorge	98	Corn Field
	São Jorge	10	Citrus Orchard
	Flores	96	Corn Field
Flores	45	Citrus Orchard	
<b>Total</b>		<b>477</b>	

Table 2. Modalities observed for each trait considered as a discriminate variable in the factorial multiple correspondence analyses, along with respective codes.

Variable	Code	Variable Description
Brown mark pattern observed laterally in the jaw	ST1	Absent or punctiforme mark located in the upper portion of the jaw insertion
	ST2	Mark stretched back by a thin line, able to reach half jaw
	ST3	Thin line
	ST4	Mark as wide as jaw stretching from the insertion point of the jaw until at least half, or even all of the jaw
Shape of the brown mark in the cheek	MG0	Mark absent
	MG1	Punctiform mark
	MG2	Wide mark close to the eye, stretching forwards in a diffuse way
	MG3	Square mark
Number of black silks on one side of the pronotum	PR1	Absent or brownish
	PR2	From 1 to 10 silks
	PR3	From 10 to 20 silks
	PR4	Over 20 silks
Size and colour of the coastal edge silks	CO1	Short and brownish silks
	CO2	Long silks transparent or brownish (silk wide = 10-16 *10-2 mm)
Pleural membrane ( $\mu$ )	MP0	Colour structure absent
	MP1	Colour structure present in the second sternite
	MP2	Colour structure present beyond second sternite and well visible in the first
Presence and distribution of black silks in the abdominal sternites	AB0	Black silks absent
	AB1	Silks spread over one or more sternites
	AB2	Silks uniformly spread over one or two sternites
	AB3	Silks uniformly spread in two or more sternites

The interpretation of the results was based upon the analysis of the graphic obtained by the application of the multiple factorial correspondences technique (Foucart 1997). In order to introduce accuracy into the graphic interpretation, an automatic classification has been applied to the point-coordinates matrix, on the first three axes. This ascendant hierarchical classification utilises a euclidian distance; it takes the weighted average of distances, as a joining criterion. The classification was made initially on the basis of ten Series.

## RESULTS

The graphic factorial multiple correspondence analysis (Fig. 2) ran on the initial data matrix, locates individuals and modalities of

morphological variables along the four quadrants. The first three axes develop 39 % of the inertia. Their patterns are outlined below.

**Series 1:** consisting of three variable modality points, ST1, CO2 and MG1; as such, grouped with Series 2.

**Series 2:** almost exclusively the Neotropical *C. externa* (Hagen, 1861) and the Nearctic *C. rufilabris* (Burmeister, 1839), the two foreign species, together with three individuals of *C. mediterranea* (Hölzel, 1972). This analysis did not account for "brown veins" and "red cheeks", two variables which would separate *C. externa* from *C. rufilabris*; besides, the variable "claw shape" would discriminate *C. mediterranea* (Thierry et al. 1998).

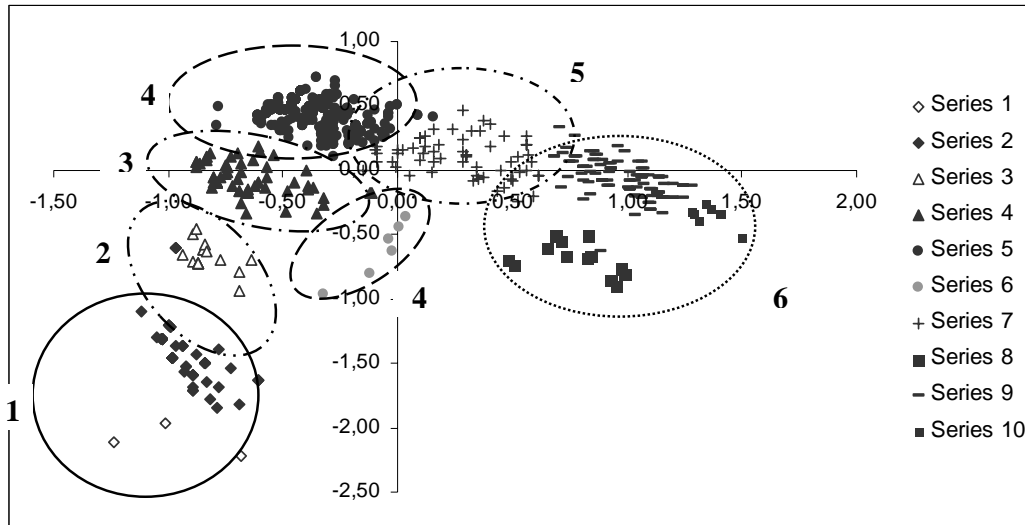


Fig. 2. Positioning of *Chrysoperla* spp. individuals according to the modalities of the discriminate morphological variables, into ten series. Numbers on circles correspond to the six groups obtained from the ascendant hierarchical classification. Note: see text for further explanations.

**Series 3:** exclusively *Ch. carnea* (nomenclature according to Canard & Thierry 2007)

**Series 4:** a majority of *C. carnea* but also grouping some Azorean individuals, morphologically close to this continental European species.

**Series 5:** made up of *C. affinis*, plus 9 undetermined individuals.

**Series 6:** assemblage of individuals morphologically close to *C. affinis*.

**Series 7:** includes some *C. mediterranea* and morphologically unidentified individuals. This series groups the more atypical individuals of the analysis, implying that there may be a morphological variability within the live material observed, i.e. beyond the limits of the statistical model used. However, it should be noted that these results are still relatively satisfactory since this series is made up of less than 10 % of the total, with less than 5 % of atypical individuals in the overall assemblage.

**Series 8, 9 and 10:** exclusively *C. lucasina*.

A dendrogram (not shown) resulting from the ascendant hierarchical classification, grouped the ten series, on the basis of the factorial analysis into six groups, truncated in Series 6 and 10, respectively.

**Group 1** (Series 1 and 2): *C. externa* and *C. rufilabris* are combined within this group due to the absence of a specific criteria for their separation.

**Group 2** (Series 3): continental European specimens of *C. carnea*.

**Group 3** (Series 4): group difficult to interpret since it contains individuals with a weak melanisation; it includes individuals of *C. carnea*, together with 16 individuals from the Azores. Given the morphological resemblance between *C. agilis* and *C. carnea*, the Azorean specimens belong probably to *C. agilis*, due to the absence of specific traits discriminating for this species.

**Group 4** (Series 5 and 6): assembles almost all *C. affinis* specimens captured in mainland

Europe, together with 68 Azorean individuals. This gives rise to two hypotheses: either the species is also present in the archipelago, although not yet identified in the current courtship song survey, or the individuals are *C. agilis* which, due to the set of criteria used, could not be separated from this sibling species.

**Group 5** (Series 7): few individuals made up of atypical specimens, together with a few individuals of *C. mediterranea*.

**Group 6** (Series 8, 9 and 10): assembles all the *C. lucasina* captured in the Azores, Madeira and Portugal mainland.

Both factorial and hierarchical analyses clearly separate *C. lucasina* from the other species; they join the insular populations (Azores and Madeira), with those from Europe mainland. Henry et al. (1996) described a unique courtship song for this species and recognised it for the insular green lacewing populations of the Portuguese archipelagos (Ventura et al. 2005).

The second species found in the archipelago shows the continental European *C. agilis* song type (Henry et al. 2003). Although no morphotype was found, given its resemblance with other species of the complex, namely *C. affinis* and *C. carnea*, its courtship song was also identified in some specimens sent to Dr. Charles Henry. Thus, Azorean specimens belonging to this species are probably distributed between Groups 3 and 4. Another hypothesis would be the presence in the Azores of both *C. affinis* and *C. carnea*, but not yet confirmed in the current courtship song survey.

## DISCUSSION

As written above, Azorean entomofauna consists predominantly of Palaearctic elements, and the Neuroptera order does so, represented only by Chrysopidae and Hemerobiidae. These families are responsible for high species richness in mainland Europe (Aspöck & Hölzel 1996) which is probably the reason why they are the only families presently occurring in the archipelago (Aspöck et al. 2001; Borges et al. 2005). But there is a greater diversity within the family

Hemerobiidae, so far, with five known species, of which, one is endemic (*Hemerobius azoricus* Tjeder, 1948) (Ohm 1973; Borges et al. 2005).

The younger geological age of the archipelago explains partially the lower Azorean species richness compared to Madeira (Aspöck et al. 2001). In spite of a greater probability for insects to reach insular ecosystems, species of Chrysopidae have a weak flying ability, which makes them very dependent upon the assistance of dispersal agents such as wind. In island colonisation experiments, Simberloff & Wilson (1969) reported that arthropods with weak flight ability are among the firsts to settle down and to reach high population densities, due to a trade-off between reproductive success and strong wings and muscles (Zera & Denno 1997).

In the common green lacewings, dispersion migratory flights take place in the first nights after emergence, being able to progress as far as 40 km in only one night (Duelli 1980). Most females not yet pregnant show ability to make ascension in altitude (Kokubu & Duelli 1986) and to abandon their original habitat before starting to lay eggs. These aspects make them good long-distance colonisers (Duelli 1984).

The wind regime in the Azores does not have a defined pattern (Morton et al. 1998), although there are frequently strong winds with an east-western direction (Chapman 1954), and a south-western direction (Agostinho 1938b; Fernandes 1985). In summer, when these multivoltine species are reproductively active, there is a predominance of north-easterly winds that increase from January to July, according to the location of the Azorean anticyclone. This may confirm the Palaearctic origin of the wind-born Azorean Chrysopidae populations. Madeira archipelago may have served as a “stepping-stone” to the Azorean colonisation due to its geographic proximity (Balletto et al. 1990). Ohm (1973) reports the presence of *Hemerobius azoricus* in the Azores, whose closely related *taxons* inhabit the Madeira and Canary islands, supporting the possibility of a biogeographical link between the Azores and the other mid-Atlantic islands.

Similarities amongst insular populations and those of the West-Palaearctic Region were confirmed through the present morphological

discriminant factor analyses. Conversely, the Nearctic *Chrysoperla rufilabris* and the Neotropical *Chrysoperla externa* were clearly separated from the other specimens. They were joined together in a separate cluster, as there were no sufficient morphological criteria on which to separate them. The study of insular species courtship songs led Ventura et al. (2005) to report the presence of *C. agilis* and *C. lucasina* in the Portuguese archipelagos of Azores and Madeira. In morphological terms, the present analysis was efficient in characterising *C. lucasina* as a valid taxon associating insular and continental specimens. However, the absence of a proper set of criteria to characterise morphologically *C. agilis*, together with its resemblance to other two species of the complex (*C. affinis* and *C. carnea*), prevents its clear identification. Nonetheless, a thorough analysis of the graphical presentation (Fig. 2), shows that the two series/groups containing the Azorean individuals which lie morphologically close to *C. carnea* (Series 4; Group 3) and to *C. affinis* (Series 6; Group 4), are located closely to the continuum between Group 2 (continental *C. carnea*) and Series 5 of Group 4 (continental *C. affinis*). A reasonable explanation is that these specimens belong to *C. agilis*, indicating the absence of continental European specimens in the related samples.

The “*carnea* complex” scenario within Europe may be presented as following. *Chrysoperla lucasina* has an Atlanto-Mediterranean distribution (Thierry et al. 1996) and *C. agilis* occurs in southern Europe and Mediterranean islands, extending up to the south of Switzerland (Henry et al. 2003). These species are associated with the same climate and the same habitats. Le Grand (1984) has suggested that most of the entomological fauna brought to the islands by humans originated from the Iberian Peninsula, a region where *C. affinis*, *C. carnea* and *C. lucasina* have already been recorded (Thierry et al. 1996). Consequently, there are no ecological reasons for the absence of *C. agilis* in the Iberian Peninsula. This species occurs in both Portuguese archipelagos (Ventura et al. 2005) and its presence in the Canary Islands might be possible, given the existence of adequate climate and habitat.

What about the presence in the Azorean archipelago of *C. carnea* and *C. affinis*, which exist in the Iberian Peninsula? Azorean specimens morphologically close to each of them were found in our samples (Fig. 2: Groups 3 and 4). Between the two species, *C. carnea* is the more likely to be absent from the Azores, for two main reasons:

1) *C. agilis* is cryptic with these two species, showing some intermediate characteristics. In figure 2, Group 3, containing a few Azorean individuals, separates from Group 2 where all the continental specimens of *C. carnea* are grouped. Conversely, Series 6 from Group 4 is positioned closer to the Group 3 individuals than the remaining of Group 4 (Series 5). Thus, a more defined set of criteria to characterise *C. agilis* morphotype would certainly separate this species from the others.

2) The niche preference of this species involves the canopy of deciduous forests, or urban areas (Duelli et al. 2002; Henry et al. 2002), habitats not so common in the Azores.

In contrast, *C. affinis* has been reported on herbaceous vegetation and tall grasses in open fields and croplands (Henry et al. 2002). Trouvé et al. (2002) refer it as the dominant species on agroecosystems in northern France, and Duelli et al. (2002) state that it is an ubiquitous species found from cultivated land to managed forest. Thus, there are no ecological reasons for its absence from the Azorean archipelago. The record of courtship song is a reliable method to discriminate among these cryptic species but the Cc4 song type has not yet been found in the archipelago. The sampling effort on the islands needs to be increased and a more accurate set of morphological criteria need to be selected. One should be able to clearly separate the *affinis* – *carnea* – *agilis* morphotypes. Finally, since the amount of dubious identification cases was less than 5 % of the total, the presence of hybrids in the field appears very unlikely.

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