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AN INDIVIDUAL MARKING TECHNIQUE FOR GREEN LACEWINGS (NEUROPTERA: CHRYSOPIDAE)

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The method of studying an animal population by marking some of its individuals was first used by the fishery biologist C. G. J. Petersen at the end of XIX century (Le Cren 1965), but it has been widely used, also by entomologists, starting around 1920 (Hagler & Jackson 2001). Insects can be marked individually or in large groups. Mass marking, usually in the form of a collective application of dust, paint, or dye, permits the identification of the individuals belonging to the marked group within a larger unmarked population to obtain general information. Individual marking permits the identification of a single individual to obtain additional information such as individual survival, fecundity and dispersion. Individual marking falls into 3 principal categories: (1) mutilation, (2) labeling, and (3) direct marking (Walker & Wineriter 1981).

Direct marking is accomplished using a code based on numbers, symbols, colors and positions. There are a wide variety of substances available to the researcher as potential marking materials (Hagler & Jackson 2001; Southwood 1978; Southwood & Henderson 2000). Ink has a number of important properties which make it a good marker. Some kinds of ink are durable, quick-drying, light-weight, easy to apply and cheap (Wineriter & Walker 1984).

Marking techniques and markers are not always practical for every kind of insect. As a result, entomologists must often develop unique methods depending on their needs. In the last decades many methods have been developed and a number of markers have been used, both for individual and for mass insect marking (Hagler & Jackson 2001). Until now there has not been any individual marking technique specific to Neuroptera, whereas a mass insect marking technique applicable also to Neuroptera is described by Horton et al. 2009.

We present a simple technique to individually mark adult green lacewings using ink. This method was developed on *Chrysoperla pallida* Henry et al. (Neuroptera Chrysopidae), but it is easily applicable to other green lacewing species when individual specimens need to be recognized.

Our laboratory colony of *C. pallida* was established with females collected by hand net in orchards near Sassari, north Sardinia, Italy. The rearing method used was a simplified version of the one described by Pasqualini (1975). Adult rearing cages were plastic cylinders closed at both ends with tulle netting on which the females laid eggs. Food was honeybee pollen loads. Lacewings had continuous access to water presented in a wet cotton plug placed on the tulle netting at the top of the container. Eggs were isolated and larvae were reared individually, in small plastic containers, in order to avoid cannibalism. Larvae were fed twice a week with *Tenebrio molitor* L. (Coleoptera Tenebrionidae) larvae. The colony was kept at 20 ± 1 °C temperature, 70 ± 10% RH and 16:8 h L:D.

In order to apply this marking technique, the specimens had to be immobilized by anesthesia with ethyl acetate. Ethyl acetate as an anaesthetic does not adversely affect lacewing fecundity, fertility and survival (Loru et al. 2010). We applied a durable, quick-drying ink with Superfine (0.4 mm) OHPen Universal Permanent Stabilo® (Heroldsberg, Germany) by placing dots on the surface of the forewings (Fig. 1). Similar results could be obtained with Point 88 Stabilo® marketed in USA by MacPherson's (Emeryville, California). In particular, dots were made in the cells between the pseudomedia and pseudocubitus veins. Our marking code is based on a binary numerical system. The number of cells utilized depends on the number of individuals one needs to mark. Four cells of only one wing allow us to mark 16 specimens. If both wings are alternatively used, the number of uniquely markable individuals becomes 32. Thus marking both wings, increasing the number of marked cells, utilizing more colors and using sex as a discriminating factor are other ways to enhance the possible number of individuals each marked uniquely (Fig. 2).

Two treatments, marked and control, were used to determine if this marking technique has negative influences on individual survival. Each treatment consisted of 24 newly emerged specimens subdivided into 4 groups of 3 male and 3 female peers. Specimens were reared in the same manner as the laboratory colony. The control individuals were placed directly in the rearing units, the marked ones also were placed there,

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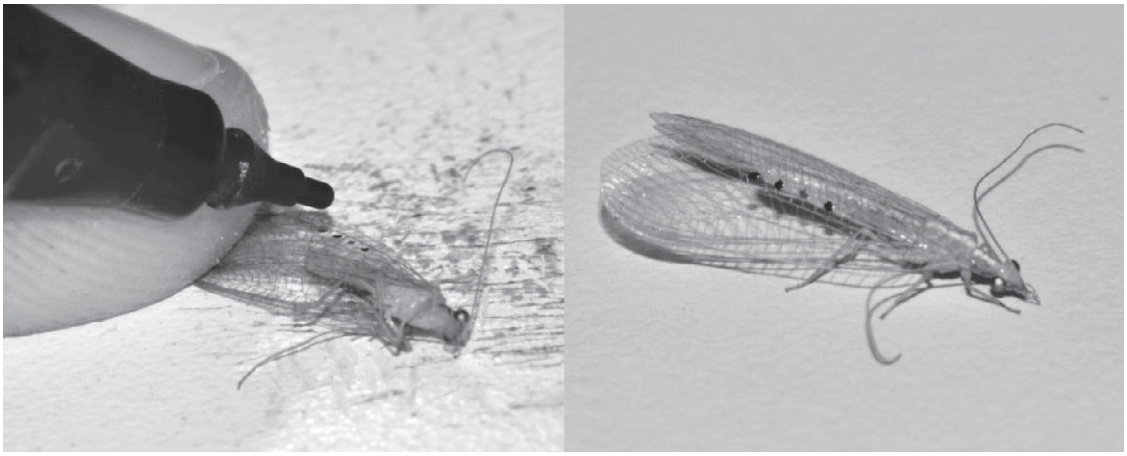

Fig. 1. Marking of an anesthetized specimen (left); marked green lacewing (right).

but about an h after marking when they had completely recovered from anaesthesia. Individuals were checked every 24 h for a 15 day period to record any deaths and then reared until their death in order to register their lifespan.

Zero and 1 specimen, respectively, died in the marked and control groups within 15 days. According to the Mann-Whitney-Wilcoxon test (W = 2.5, df = 1, $P \geq 0.05$) the mortalities in these 2 treatments were not significantly different.

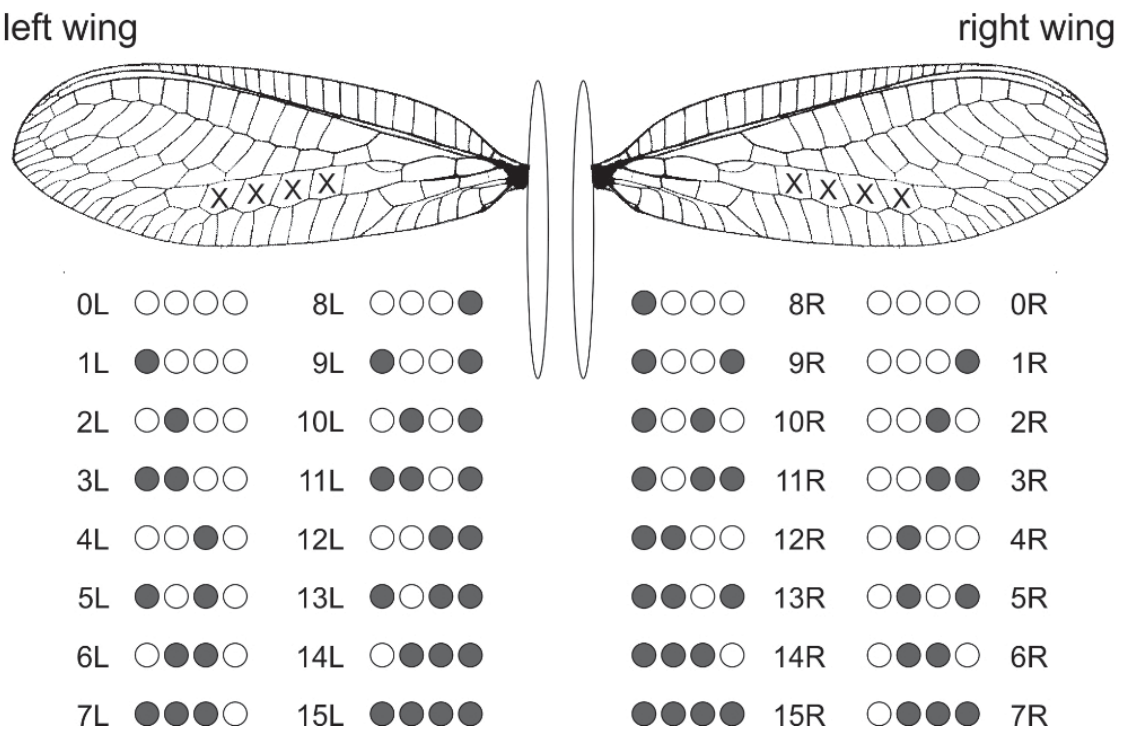

Fig. 2. Marking code. Using a simple binary code (2 digits corresponding to colors: light = 0, dark = 1), 4 cells provide 16 combinations (number of digits$^{\text{number of cells}}$, *viz.* 2^4) that could be multiplied by the wings (right and left) and the sex (male and female) reaching 64 (16 × 2 × 2) individual marked specimens. Increasing the number of cells (the 2 cells adjacent to the ends of the line) this value can be doubled or quadrupled. Varying slightly the number of colors (= digits) it is possible to reach a higher value: $3^4 = 81$, $4^4 = 256$, etc. Combining number of cells and colors it is possible to obtain a wide range of combinations (around a thousand) without overcomplicating the code. Spots must be made on the dorsal side of the wing; x = cell usable for marking.

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Lifespans were 88.9 ± 25.0 (mean ± SD) and 86.0 ± 21.3 (mean ± SD) days for marked and control groups, respectively. These lifespans were not significantly different according to the Mann-Whitney - Wilcoxon test (W = 24.0, df = 1, $P \geq 0.05$).

This new marking method does not have any obvious influence either on lacewing survival within 15 days from treatment (excluding the influence of traumatic manipulation or acute poisoning) or on their lifespan. This method is reliable, cheap, and easy to apply and to decode. It is particularly suitable for use in laboratory experiments.

Summary

We present a new technique developed to uniquely mark individual adult green lacewings (Neuroptera Chrysopidae) with a durable, quick-drying ink applied by a very fine tipped pen. This marking method is particularly suitable for use in laboratory experiments. It is reliable, cheap, and easy to apply and to decode.

Key Words: anesthesia, code, ethyl acetate, ink, unique, wing cell, pseudocubitus vein

Resumen

Se presenta una nueva técnica desarrollada para marcar individuos específicos de adultos de crisópidos (Neuroptera: Chrysopidae) utilizando tinta. Este método de marcar es particularmente adecuado para su uso en ensayos de laboratorio. Es fiable, barato y fácil de aplicar y decodificar.

Palabras Clave: código binario, acetato de etilo, tinta, marcado directo, marcar individuos específicos

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