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Individual photographic identification based on unique colour pattern of the thorax of *Acherontia atropos* (Linnaeus, 1758) (Lepidoptera: Sphingidae)

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Abstract

Natural marks have increasingly been used as a tool for individual identification. One of the most popular techniques used by natural marks as an individual recognition tool is photo-identification. Photo-identification is a non-invasive alternative to traditional marking, which allows individual recognition of species through time and space. In this study, the APHIS (Automatic Photo Identification Suite) software has been evaluated as software capable of identifying individuals of *Acherontia atropos* (Linnaeus, 1758). The SPM (Spot Pattern Matching) and ITM (Image Template Matching) procedures were tested and found to achieve 100% success of individuals recognition. Thus, for the first time in a Sphingidae, the colour pattern of the dorsal part of the thorax of *A. atropos* is demonstrated to represent a suitable natural mark for individual recognition.
KEY WORDS: Lepidoptera, Sphingidae, *Acherontia atropos*, individual marking techniques, natural marks, APHIS, Balearic Islands, Spain.

Identificación individual fotográfica basada en el patrón de coloración único del tórax de *Acherontia atropos* (Linnaeus, 1758) (Lepidoptera: Sphingidae)

Resumen

Las marcas naturales se utilizan cada vez más como herramienta de identificación individual. Una de las técnicas más utilizadas con las marcas naturales y como herramienta de reconocimiento individual, es la fotoidentificación. La fotoidentificación es una alternativa no invasiva al marcaje tradicional, que permite el reconocimiento individual de especies a través del tiempo y espacio. En este estudio, el software APHIS (Automatic Photo Identification Suite) ha sido evaluado como software capaz de identificar individuos de *Acherontia atropos* (Linnaeus, 1758). Se probaron los procedimientos SPM (Spot Pattern Matching) e ITM (Image Template Matching) logrando el 100% de éxito de individuos reconocidos. Así, por primera vez en una especie de Sphingidae se demuestra que el patrón de color de la parte dorsal del tórax de *A. atropos* constituye una marca natural adecuada para el reconocimiento individual.
PALABRAS CLAVE: Lepidoptera, Sphingidae, *Acherontia atropos*, técnicas de marcaje individual, marcaje natural, APHIS, Islas Baleares, España.

Introduction

Population ecology studies provide basic information to understand population dynamics and are fundamental for the study of conservation, management and control of endangered and invasive species and species of economic importance. Often, these studies are based on capture-mark-recapture (CMR) techniques to estimate population parameters (SOUTHWOOD & HENDERSON, 2009). To develop properly CMR techniques it is necessary to use individual marking techniques that allow recognising individuals in future capture events. These marking techniques can be artificial or natural, or a combination of both (MANN *et al*., 2000). One of the most commonly used techniques are the artificial ones (OOSTHUIZEN *et al*., 2010; SILVY *et al*., 2012). Several types are used according to the zoological taxa. Specifically, among Lepidoptera these conventional marking techniques consist on manual assignment of an alphanumeric code on the discal cells such as the study done with *Morpho sulvkowskyi* (Kollar, 1850) (Nymphalidae) (PRIETO *et al*., 2005) or with *Boloria acrocnema* Gall & Sperling, 1980 (Lepidoptera: Nymphalidae) (GALL, 1984), the use of fluorescent dust with *Plutella xylostella* (Linnaeus, 1758) (Plutellidae) (CAMERON *et al*., 2002) or the use of tags with a numbered gummed label used in migrating species such as *Danaus plexippus* (Linnaeus, 1758) (Nymphalidae), and the use of commercial quick-drying ink to estimate population parameters (BROWER, 1962; KNIGHT, 1999).

These artificial marking techniques work efficiently, as most of the brands and labels used in animals, are fast and easy to apply (HIGGINS *et al*., 1997). However, artificial marking techniques can be harmful. Some studies pointed out those artificial marking techniques can influence on the capture probability by under or overestimating the demographic parameters as a result of studying a researcher-influenced population rather than the real population. For instance, SINGER & WEDLAKE (1981) found that recapture probability in *Graphium sarpedon* (Linnaeus, 1758) (Lepidoptera: Papilionidae) was affected by the style of capturing and marking. On the other hand, a study carried out on *B. acrocnema* the marking effect generated a large positive bias in population size estimates with no differences in mortality rate (GALL, 1984). Furthermore, a wrong dose of fluorescent dust as a marking technique used in *P. xylostella* (Linnaeus, 1758) resulted in a 7 % of additional mortality (CAMERON *et al.*, 2002). MORTON (1982) stated that the marking process itself is not harmful according to the studies with on *Melanargia galathea* (Linnaeus, 1758) (Lepidoptera: Nymphalidae). Nevertheless, the repeated disturbance of individuals because of the capture, marking and recapture events would have negative effects (MORTON, 1982; MURPHY, 1987).

Therefore, in recent years, natural marks have increasingly been used as a tool for individual identification, especially in vertebrates (STEVICK *et al*., 2001). One of the most popular techniques that use natural marks, as an individual recognition technique, is photo-identification. Photo-identification is a non-invasive alternative to traditional marking techniques, which allows individual recognition (DELANY, 1978; SACCHI *et al*., 2007). In this sense, photo-identification has become an important tool, since it does not influence both upon the vertebrate and the invertebrate species in the same way artificial marking techniques do (GALL, 1984; SPEED *et al*., 2007).

Photoidentification technique requires an image of a fixed part of an organism, which is common to all individuals of the same species, but which must be unique to the individual to study (MOYA *et al*., 2015). In addition, not all species are suitable for photo-identification; those that do not have unique natural patterns cannot be recognised individually. Therefore, the photo-identification is restricted to those species with different colours, spots, or marks among the individuals within a population (MOYA *et al*., 2015). These techniques have been widely used in vertebrates (DUNBAR *et al*., 2014; LANGTIMM *et al*., 2004; SHERLEY *et al*., 2010; ZHENG *et al*., 2016). However, only a few studies used photo-identification with invertebrates. Some of these studies applied it in octopuses (HUFFARD *et al*., 2008), starfishes (CHIM & TAN, 2012), sea cucumbers (RAJ, 1998), crustaceans (FRISCH & HOBBS, 2007) or insects (DÍAZ-CALAFAT *et al*., 2018).

In this study, the death's-head hawkmoth, *Acherontia atropos* (Linnaeus, 1758) (Lepidoptera: Sphingidae), has been chosen as a potential species suitable for photo-identification. This species is native to Africa and southern Europe and is characterised by migrating to northern Europe year after year (KITCHING, 2021). Adult longevity of *A. atropos* was estimated in captivity around 18-30 days in males and 25-40 days in females, which reduces the possibility of variation of natural marks (ZAGIRUNSKII *et al*., 2013). In addition, it has a descriptive skull-shape marked on the thorax that makes it unique in the regions where it occurs. According to this trait we propose to analyse and test if the skull-shape mark can be used to identify individually the specimens of a population because of the apparent variation in shape, colour, and size of this skull. Therefore, the aim of this study is to assess, for the first time in a moth species, whether the colour pattern of the dorsal part of the thorax of *A. atropos* is suitable for individual recognition.

Materials and methods

To test if the skull shape is different among the specimens of *A. atropos* different specimens were analysed and performed a photo identification test in a simulated closed artificial population. 79 *A. atropos* individuals were collected as dead specimens from honeybee hives in Manacor municipality (Mallorca, Balearic Islands, Spain) during the year 2019. It is common to observe dead specimens of *A. atropos* around beehives, since honey bees kill intruders as *A. atropos* which try to enter the hive (BRUGER, 1946). Once collected, they were preserved in labelled paper bags and stored up frozen for further analysis. Next, all the specimens were extended in entomological drawers and deposited at the Interdisciplinary Ecology Group entomological collection (University of the Balearic Islands, Balearic Islands, Spain).

To recognise each captured specimen between three and five different photographs of the dorsal part of the thorax were taken. Each photograph was labelled with a unique alphanumeric code for each specimen differentiating each picture. The photographs were taken with a digital camera (Canon EOS 50D model) and a natural light source. Then, the three best photographs of each specimen were chosen, and the photographs were evenly distributed into 17 different computer folders (16 computer folders with 14 images and one computer folder with 13 images) to perform 17 CMR sessions. Subsequently, all the pictures were analysed to test if photo-identification of the dorsal part of the thorax is an appropriate technique by using two different procedures within APHIS software (MOYÀ *et al*., 2015).

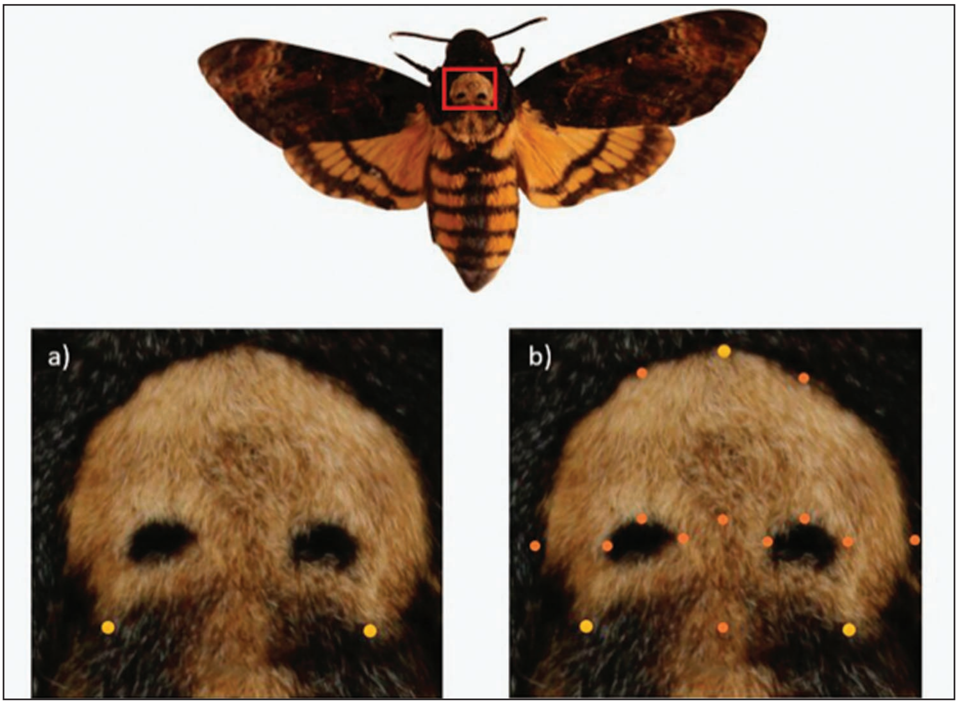

Figure 1.– Set of reference points (yellow points) and accessory points (orange points) selected in the dorsal part of the thorax *Acherontia atropos* when using the ITM (a) and SPM (b) procedure of the APHIS program.

APHIS software

To check if the colour pattern of the natural marks of the dorsal part of the thorax of *A. atropos* is appropriate to perform the photo-identification, APHIS program was used as free software of picture matching (MOYA *et al*., 2015). Unlike other photographic coincidence programs such as I3S Manta (DEN HARTOG & REIJNS, 2008), Wild-ID (BOLGER *et al*., 2012) and Extract Compare (PATERSON *et al*., 2013), APHIS allows to choose up to 100 possibly candidates, making it less likely to make a photographic matching mistake. In addition, APHIS can process hundreds of photographs at the same time and users can select two types of matching procedures: an interactive and newly created SPM (Spot Pattern Matching) and an automatic ITM (Image Template Matching) based on the I3S method (MOYÀ *et al*., 2015).

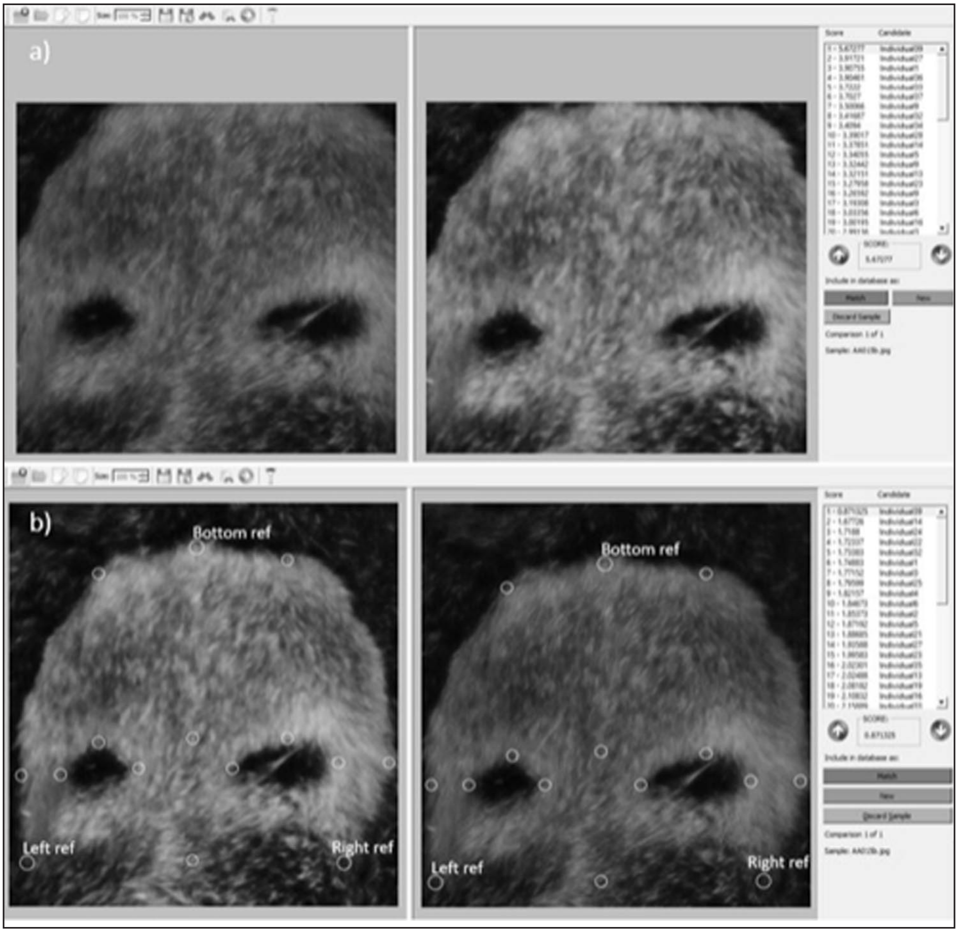

Figure 2.– Examples of photo recaptures of *Acherontia atropos* detected using the ITM (a) and SPM (b) procedures. In both cases, the matching recapture was the first candidate in the candidate list generated by the APHIS program.

By using the ITM procedure, only two reference points are needed, which were chosen on both the left and the right side of the basis of the skull shape (Fig. 1a). On the other hand, for the SPM procedure, three reference points and a minimum of 12 accessory points are needed, so the two points made in the ITM procedure were chosen together with a third reference point and 12 accessory points (Fig. 1b). Once the images are compared, the software provides the most likely candidates ranked by a similarity coefficient for each introduced photograph. It highlights that similarity coefficients are estimated differently. In the SPM procedure the more similar the two compared individuals were the lower the similarity coefficient was. On the other hand, in the ITM procedure the more similar the two compared individuals were the higher the similarity coefficient was (Fig. 2).

Analysis of data

All collected data was organised and processed with a spreadsheet. Subsequently, the statistical program R (R CORE TEAM, 2017) was used to analyse the differences between both procedures. Means, standard deviations, minimums, and maximums of the recapture candidate positions in each procedure were estimated. A Mann-Whitney U distribution (non-parametric) test was used to test differences of mean candidate position in ITM and SPM procedure, since data were not distributed normally, and variances were not homogeneous. Significance level was set to $\alpha < 0.05$.

Results

For the realization of the photo-identification, by means of the SPM and ITM procedures of APHIS software, a total of 79 specimens of *A. atropos* were analysed. By using the ITM procedure all recaptured specimens were matched correctly, and they were individually recognised. 98.73% of the recaptured specimens matched with the first candidate position, and up to 99.37% of the recaptures were established within the performance threshold proposed between the first and the fifth candidate position of recapture. Only one specimen was positioned beyond the threshold, occupying the sixth recapture position, representing 0.63% of the total recaptures obtained. The mean of the similarity coefficients, of all recaptured specimens, was 5.434 ± 0.556 with a minimum of 2.483 and a maximum of 5.920 (Fig. 3. Table 1).

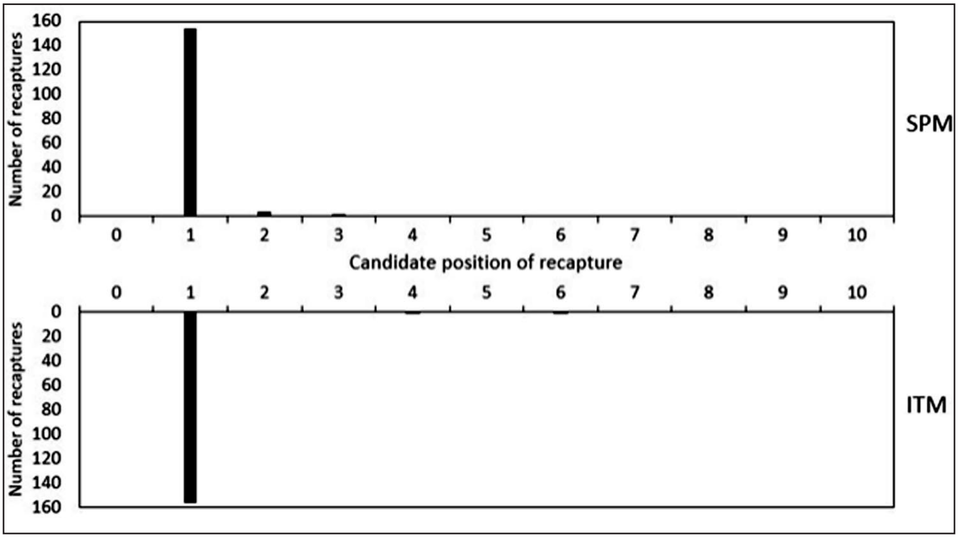

Figure 3.– Distribution of the number of photo recaptures and its candidate position of recapture of *Acherontia atropos* by using both ITM and SPM procedures within APHIS software.

In contrast, by using the SPM procedure all recaptured individuals were matched correctly and they were individually recognised. Ninety-seven (97.47) % of the recaptured specimens matched with the first candidate position, and all the recaptures were established within the performance threshold proposed between the first and the fifth candidate position of recapture. Mean of the similarity coefficients of all recaptured individuals was 0.952 ± 0.261 with a minimum of 0.411 and a maximum of 1.666 (Fig. 3; Table 1).

Table 1.– Number of photo recaptures of *Acherontia atropos* ordered according to their candidate position given by APHIS software and the ITM or SPM procedures with the mean of its similarity coefficient (mean ± typical deviation, range). *It is a single individual so the average, typical deviation is not provided.

	SPM		ITM	
	N of captures	Similarity coefficient	N of captures	Similarity coefficient
1st position	154 (97.47 %)	0.946 ± 0.260 (0.411-1.666)	156 (98.73 %)	5.465 ± 0.482 (3.101-5.920)
1st-5th position	158 (100 %)	0,952 ± 0.261 (0.411-1.666)	157 (99.37 %)	5.452 ± 0,505 (3.101-5.920)
> 5th position	-	-	1 (0.63 %)	2.483*
Total	158 (100 %)	0,952 ± 0.261 (0.411-1.666)	158 (100 %)	5.434 ± 0.556 (2.483-5.920)

Recapture of specimens presented a candidate position of recapture that oscillated between the first and the third position (= 1.032 ± 0.209; n= 158) by using the SPM procedure and between the first and sixth position (= 1.051 ± 0.463; n= 158) by using the ITM procedure. No significant differences were obtained in the average number of the recapture positions between the ITM procedure and SPM (M-W U test, U= 12328,00; p= 0.424) (Fig. 4).

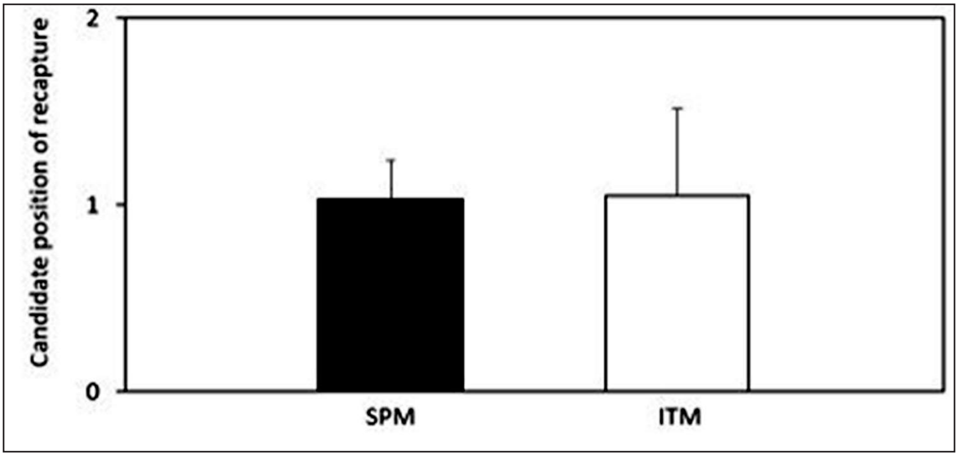

Figure 4.– Candidate position at photo recapture (mean ± typical deviation) of *Acherontia atropos* according to both ITM and SPM procedures using APHIS software.

Discussion

Photo-identification techniques are scarcely used in insects. Some studies on photo-identification has been done with Coleoptera, such as the Cerambycid beetle species *Rosalia alpina* (Linnaeus, 1758) with the colouring pattern of their elytra to recognize them individually (CACE *et al*., 2013; ROSSI DE GASPERI *et al*., 2016) or the Lucanidae beetle species *Lucanus cervus* (Linnaeus, 1758), which the amount of denticles and their position in the jaws vary individually (ROMITI *et al*., 2017). As far as known, photo-identification has only been used in one other lepidopteran *Heliconius charithonia* (Linnaeus, 1767), which the pattern of colouring of the edge of the hindwings was used to recognize them individually (DENIS & FLORES, 2017).

According to our data, positive results on correct matching with *A. atropos* specimens provided higher values of similarity coefficients than other studies with different taxonomical groups by using APHIS software (MOYA *et al.*, 2015). Even, the obtained similarity coefficients from both SPM and ITM procedures were far higher when compared to other studies carried out with other insects using the same software (e. g. DÍAZ-CALAFAT *et al*., 2018).

The skull-shape located onto the thorax seems to be quite different and variable among all the analysed specimens, and it could be used as a natural mark for individual recognition in *A. atropos*. That is, the colour pattern of the dorsal part of the thorax, and its use for photoidentification provides a reliable tool to identify specimens of this species and it can become an alternative to conventional marking techniques. Consequently, this is the first time ever that it is demonstrated in any moth species. Other species of the genus *Acherontia* [Laspeyres], 1809 such as *A. lachesis* (Fabricius, 1798) and *A. styx* Westwood, 1847 have similar skull-shape onto the thorax, thus suggesting that photoidentification may be a useful individual technique within the genus.

The potential use of photoidentification technique in *A. atropos* is especially important in a species which is considered a natural enemy and a pest of honeybee with economic relevance (HAMIDA, 1999). A high population of this moth species can rapidly deplete the stores in a colony by stealing the honey, though the disturbance it creates is much more serious since it can lead to the abandonment of the queen and worker bees from the hive (SARWAR, 2016). The use of natural marks such as the skull-shape on the thorax would allow one to stablish monitoring schemes of natural populations of *A. atropos* and expand the knowledge of its population dynamics. Specifically, this technique would allow estimating population parameters of *A. atropos* by carrying out CMR studies and its interaction with honeybees.

Further studies should be performed in the future to establish the stability of the natural mark throughout the lifetime of *A. atropos*, since within moth and butterfly species scales are lost because of their activity. On the other hand, new studies are needed to find out if natural marks from other species of Lepidoptera or even insects can be used to recognize individuals within a population, and whether they could be used for photo identification techniques. If positive results arise, the development of population ecology studies based on CMR techniques will uncover some population estimates such as population size or survival rates according to sex, which nowadays are completely unknown for many species of moths and butterflies.

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