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Non-lethal way of securing insect specimens in the field for morphometrics studies

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Abstract

For many rare or protected species, it is unwise to collect the large series of specimens necessary for morphological studies. We present here a method for securing live specimens in the field to store images in a standard way. A portable stand and a digital camera allow the storage of standardised pictures at reasonable price. Individual insects are kept still between transparency sheets for photography, and are released afterwards. Freely available software now provide the necessary means to extract morphological information, and to carry out subsequent statistical analyses. This method was originally designed for butterflies, but it should be suitable to other large winged insects.

Keywords : morphometrics, photography, Lepidoptera, methods.

Résumé

Pour les espèces sensibles, il n'est pas souhaitable de prélever les effectifs importants nécessaires pour des études morphométriques quantitatives. Nous présentons ici une méthode permettant de photographier des spécimens de manière standardisée sur le terrain. L'utilisation d'un appareil photographique numérique compact permet de prendre de grandes quantités de clichés à prix et encombrement réduits. Les individus sont maintenus entre deux feuilles de plastique transparent pour la photographie. Des logiciels libres performants sont actuellement disponibles pour le traitement des images et les analyses statistiques subséquentes. La présente méthode a été mise au point pour des Lépidoptères de grande taille. Elle est aussi applicable à d'autres espèces, surtout de grande taille.

Introduction

For ecological studies, it may be worthwhile to record the phenotype of every individual studied, in order to investigate the relationship between any morphological trait and other variables, such as behaviour, survival or reproductive success. Digital photography now offers the possibility of recording the morphology of every individual before its release. Furthermore, in small populations, whatever their legal status, it is recommended not to collect specimens; and of course, many countries have established lists of protected species, banning their collection. These restrictions all have great impact on the recording of insects, as their identification may not be checked later. Studies of variation, central in evolutionary studies, may not be carried out without the recording of the characters of interest.

The availability of digital cameras at a reasonable price allows the easy and cheap storage of great numbers of photographs. Many entomologists have recognised that compact digital cameras allow microphotography with ease. Until recently the only ways to perform detailed morphometric studies was to kill all the necessary specimens and to bring them to the lab,

either to study them directly through a binocular microscope (e.g. DESCIMON & RENON, 1975a and b) or to store digital images taken by a video camera (WINDIG, 1992). As an exception, BREUKER (2002) made conventional photographs of live specimens in a studio before releasing the studied specimens; the photographs were then scanned in order to take measurements with an image analysis software.

We have designed a portable stand which allows photographs to be taken in a standard way in the field. Compact digital cameras are generally much lighter than standard reflex cameras, and the use of the play function allows the researcher to check the quality of the pictures immediately after they have been taken. Morphometric studies may be carried out later, using image analysis software, some of which are freely available.

Methods

We have designed a small wooden stand (fig.1), with two boards at a right angle to each other. A small flashgun stand (easily available from any camera shop) is attached to the longer one, with the camera (in our case a Nikon Coolpix® 990) attached to the stand. The key point is to set the specimen parallel to the detector array of the camera, and to have its centre directly in front of the camera, to avoid parallax problems. The specimen should be placed exactly in front of the front lens, and the long board should be long enough so that pictures are taken with the medium or long focal length of the zoom. A check of the distortion effect should be carried out by taking pictures of graph paper and measuring distances between points at regular intervals from the centre to the edge; once this distance deviates from the expected one, the distortion zone of the lens/focal length is entered. The specimen should be kept within the non-distorted part of the picture (ZELDITCH *et al.*, 2004).

For photography, each butterfly is maintained between two sheets of plastic film (overhead projector film transparencies), with a hole allowing free movement of the head, the body and the legs of the individual. These two sheets are held together by a piece of cello tape as a hinge. It is worthwhile taking several transparency sheets into the field, in order to replace damaged or dirty ones. They may be washed with alcohol and non fibrous material, such as the ones sold to clean computer screens. For photography, each individual butterfly is maintained by its wings between the two sheets. Clips are placed on each side of the butterfly to keep the plastic folder closed. The specimen is maintained against a white background, with a hole for the body and legs. The butterfly is then photographed together with a standard for length, such as a piece of ruler, and if degrees of darkness are of interest, standards of black, white and grey.

Most digital cameras allow users to check the quality of the pictures. The important part, beside focus, is to avoid any overexposed area due to light reflection on the transparency sheet: the play function of many cameras shows such areas as blinking. If the overexposed area covers part of the subject, a second picture is taken with a different angle to the source of light, or with an

Fig. 1. The stand in the field, with a specimen of *Parnassius mnemosyne* being photographed.

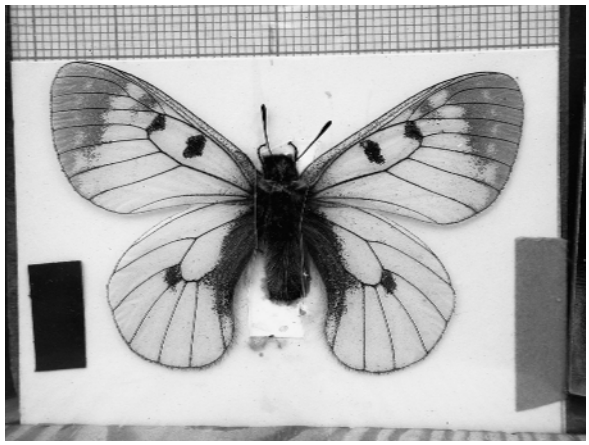

Fig. 2. An example of picture taken in the field. Note the white background to the specimen, necessary if automatic detection of perimeters of pattern elements are to be performed on the image. The clips on both sides of the specimen keep the plastic folder closed.

appropriate shade. Pictures are usually taken with the timer of the camera, in order to avoid vibrations caused by pressing the shutter.

If a series of individuals are to be photographed from a given population, these are captured and temporarily stored in glassine envelopes in a box stored in the shade. The butterflies are then retrieved one by one, and photographed before being marked and released. The marking with a permanent marker, such as Stabilo® series S, allows the identification of individuals already photographed (EHRLICH & DAVIDSON, 1961). Individual marking are also the key to population studies (DESCIMON & NAPOLITANO, 1990 ; SCHTICKZELLE *et al.*, 2003).

We have tried this equipment with Papilionid species such as *Parnassius apollo, P. mnemosyne* and *Iphiclides podalirius*, and with the Pierid *Aporia craetegi*. The use of the transparency sheets seems to be feasible only with large specimens; it is unlikely to be usable for Lycaenid species. However, if only the inferior parts of the hind wings are necessary, then the transparency sheet may be used sideways with the body of the butterfly on its side. For smaller specimens, another device, such as the WINGMACHINE (HOULE, 2003), designed for *Drosophila* wings, may be more appropriate.

The most common image analysis software available now use JPEG (Joint Photographic Expert Group, *.jpg) files. In order to keep a maximum of information, we work with the image capture setting on "fine", which generates images of *ca.* 1 MB each on the Nikon Coolpix 990 camera. As on the resulting files the pixels are smaller than the scales on the butterfly wings, the images keep all the necessary pattern information. If smaller details are necessary, it may be wise to use a camera with a finer detector array.

Image analyses are taking place later in the laboratory with standard software, such as tpsDIG2, freely available from the morphometrics laboratory at the State University of New York in Stony Brook (http://life.bio.sunysb.edu/morph/), which also maintains an database of useful information to would-be morpho-metricians, including a list of software provided by researchers from all over the world. ZELDITCH *et al.*'s *Geometric Morphometrics for Biologists* (2004) is probably one of the best teach yourself morphometrics guides available, even if its mathematical notations are sometimes confusing (ROHLF, 2005).

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