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— Note on Methodology —

A RESTRAINING DEVICE TO AID IDENTIFICATION OF BEES BY DIGITAL PHOTOGRAPHY

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Abstract—We developed a simple restraining chamber to hold captured bumble bees temporarily so they could be photographed in the field using inexpensive "point-and-shoot" digital cameras. The process is quick, and the resulting "digital voucher" images allowed us to correct a substantial fraction of field identifications based on visual inspection. The system can improve the accuracy of monitoring programs in which it is undesirable to kill specimens to provide traditional vouchers.

Keywords: insect photography, bumble bee, digital voucher

INTRODUCTION

Reported population declines in some bumble bee species (Colla et al. 2012, Hatfield et al. 2014), along with range shifts driven by climate (Kerr et al. 2015), have underscored the desirability of monitoring programs for these insects. Unfortunately, most habitats contain some *Bombus* species that are difficult to distinguish without very close examination. The classical solution, killing specimens for careful microscopic analysis, may be inappropriate for various reasons. Some species are very rare, with legal protections possibly forthcoming. In our work near the Rocky Mountain Biological Laboratory (RMBL; Pyke et al. 2011), heavy collecting is also discouraged because of possible effects on other research programs on local pollination systems. Although a recent study (Gezon et al. 2015) indicates that such worries may be misplaced, concerns may well remain. In other areas, killing insects is objectionable to local religious or ethical sensibilities (Corbet & Huang 2014).

Substituting voucher photographs for voucher specimens can partially address such concerns. Recent advances in digital photography allow almost anyone to acquire acceptable photographs of large insects without expensive professional equipment. Indeed, the citizen-science monitoring program Bumble Bee Watch operates by urging amateur observers to upload digital images (http://bumblebeewatch.org/). The uploaded images can frequently be successfully identified to species by an expert (Sheila Colla, pers. comm.), although sometimes the necessary characters are not visible. Furthermore, the task of stalking a freely foraging bee to photograph it is time-consuming and frequently ends in failure. Here, we describe a simple device for briefly and harmlessly restraining live bees that are caught by net. We used this holder, and refined its design, during a major monitoring program at the RMBL in the summer of 2014.

APPARATUS AND USE

The holder comprises a cylinder and a piston, made from two telescoping sections of rigid, transparent acrylic tubing, in the dimensions indicated in Fig. 1. The open-topped cylinder has a solid bottom with a carefully sized depression that receives the bee. The piston's bottom is a clear glass photographic filter, and its open top is fitted with an internal bushing that is sized to receive the lens of a small digital camera. After a bee is transferred into the cylinder, the piston is gently lowered so that the filter imprisons the bee in the depression. The camera's lens is then inserted into the top of the piston. The holder assembly ensures consistent alignment and a constant camera-to-subject distance. We take several photographs, raising and lowering the piston between shots to allow the bee to shift position and reveal its key characters to view. The coated filter's good optical properties promote clarity. Handling time is typically less than two minutes per bee.

The piston-and-cylinder mechanism is shared with previous devices for restraining insects, in particular the "bee squeezer" described by Kearns & Thomson (2001, pp. 73-75), in which a soft foam piston presses a bee against a screen of fabric mesh. That device was optimized to immobilize bees for marking, or for removing pollen loads (Saifuddin & Jha 2014), rather than seeing them clearly. In past investigations, we have photographed specimens in bee squeezers, but the bees are distorted, and key characters are frequently obscured by the mesh.

We used inexpensive, consumer-grade, "point-and-shoot" cameras made by Nikon and Samsung. Although we

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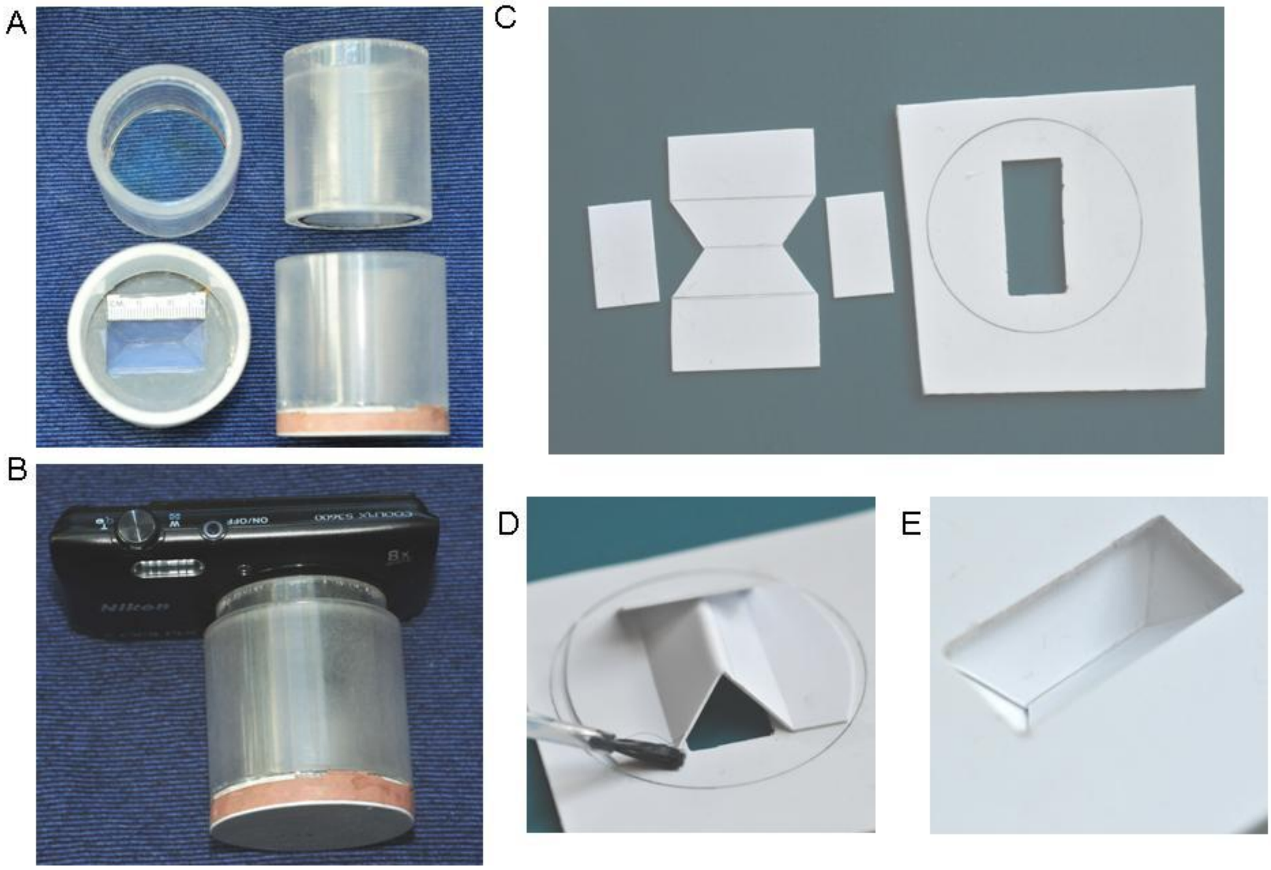

FIGURE 1. Parts of a holder device. (A) End and side views of piston and cylinder. The bee-holding declivity is visible at the bottom of the cylinder. The outside diameter of the cylinder is 50 mm. (B) Nikon Coolpix S3600 camera with lens inserted into piston. (C) Styrene sheet with layout of parts for constructing the declivity that receives the bee. (D) Assembling the declivity with liquid plastic cement. The hourglass-shaped piece is scored and folded along the lines, then cemented in place over the rectangular opening in the bottom plate. In the photo, one of the two end caps is being attached. (E) The finished declivity plate, which is then cemented to the bottom of the cylinder. To reinforce this construction and to provide a flat bottom, the entire declivity is covered with a thick layer of two-part polyester resin sold for auto body repairs (visible as a pinkish layer in side view).

initially devoted much effort to supplying additional lighting by incorporating LEDs between the filter and the depression, we abandoned those efforts when we found that we could obtain adequate images with the sunlight that entered through the transparent walls. We set the cameras to register time-and-date stamps in the images, a major aid to organizing the images later.

The principal shortcoming of this procedure was that the autofocus mechanisms of these cameras did not always capture the desired plane of focus. If the filter becomes dirty, the camera will frequently focus on that plane rather than the bee below. Furthermore, the LCD viewfinders of the cameras are hard to see in sunlight, so it is hard to verify the quality of images in the field. Smaller bees, being freer to move about, were also more difficult to manoeuvre into positions that exposed key characters. We approached these difficulties by simply taking numerous pictures of each specimen. An alternative approach would be to employ cylinders with declivities of different sizes. Frequently wiping the filters clean of dirt and pollen reduced focus problems. After discovering that restrained bees could become heat stressed if left in the sun, we tried to work quickly and to keep the holder shaded when not actually taking photographs.

Better images could surely be obtained by using higher-quality cameras with dedicated macro lenses and supplemental lighting such as a ring flash. Most higher-quality cameras also allow manual override of otherwise automated settings such as shutter speed, aperture, and focal distance. Because optimal settings were similar among all our photos, a more customizable camera would probably have improved the images further. Nevertheless, our system was not only cheaper but was small enough to carry in a pocket during strenuous fieldwork. Because the cameras were not attached to the holders, they were conveniently available to record images of the sites and the flower from which each bee was collected. Photographs of sites or flowers were especially useful as markers between photographs of consecutively caught bees. Some similar cameras have GPS features for attaching site coordinates to the images, a desirable refinement.

Camera-equipped smart phones are becoming ubiquitous, and their cameras are increasingly capable. Student assistants are likely to own smart phones that they could use, obviating the need to purchase cameras. We experimented with a handheld iPhone 4, obtaining useful images. However, a frame to hold such a camera in place would be more complicated to fabricate than our simple coaxial design in which the camera's extended lens is cradled in the centre of the piston. A phone-plus-cylinder assembly would probably be unbalanced and vulnerable to tipping over. Furthermore, different models of phones and carrying cases would need different frames.

RESULTS AND DISCUSSION

From 2 July to 9 August 2014, teams of three or four students, using three camera-and-holder assemblies, recorded 5628 images of 1456 captured bumble bees spanning 15 species. Fig. 2 shows a sample image. The collectors were trained to recognize and distinguish the local *Bombus* spp. using Williams et al. (2014) in addition to the visual "field mark" characters that have been found most useful by the RMBL research community. Following this guidance, the collectors assigned a provisional field identification of each specimen to species and caste. In checking field identifications against the digital vouchers, we confidently revised 90 (6.2%) of the field identifications and changed a further 31 (2.1%) with moderate certainty. In 83 of the cases with revised identifications, students had indicated no uncertainty about their field identifications. In 135 cases (9.3%), some doubt remained because the images were not clear enough to resolve the issue. Truly expert taxonomists could probably have resolved some of these uncertainties. We conclude that our digital vouchers substantially increased the accuracy of our identifications, and that further improvement could have been possible by taking more images of each bee.

Few bees suffered damage from being restrained. On our first day of testing, before we realized the risk of bees' overheating, we inadvertently stressed three individuals; after becoming comatose, two died, but one subsequently recovered without obvious impairments. After we adopted more careful procedures, we unintentionally killed two more bees during the entire project, for a total mortality rate of less than 0.3%.

Overall, taking digital vouchers allowed us to identify bees more accurately without much mortality or adding too much time to the fieldwork. Without the images, a substantial number of our identifications would have been incorrect. We suspect this would be true in most other localities for projects that depend on students or citizen scientists to do the field work. Of course, curating the images is very time-consuming, but that process does not cut into time in the field. The images also provide permanent records with probable future value.

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FIGURE 2. A typical photograph (*Bombus balteatus* queen), showing the time/date stamp.

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