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Stereoscopic video analysis of *Anopheles gambiae* behavior in the field: Challenges and opportunities

Nicholas C. Manoukis[a,*], Sachit Butail[b], Moussa Diallo[c], José M.C. Ribeiro[d], Derek A. Paley[e]

[a] US Pacific Basin Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Hilo, HI 96720, USA
[b] Polytechnic Institute of New York University, Brooklyn, NY 11201, USA
[c] University of Bamako, Malaria Research and Training Center, Unit of Molecular Ecology, Bamako, Mali
[d] Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA
[e] Department of Aerospace Engineering and Institute for Systems Research, University of Maryland, College Park, MD 20742, USA

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ABSTRACT

Advances in our ability to localize and track individual swarming mosquitoes in the field via stereoscopic image analysis have enabled us to test long-standing ideas about individual male behavior and directly observe coupling. These studies further our fundamental understanding of the reproductive biology of mosquitoes. In addition, our analyses using stereoscopic video of swarms of the African malaria vector *Anopheles gambiae* have produced results that should be relevant to any "release-based" method of control including Sterile Insect Technique (SIT) and genetically modified male mosquitoes (GMM). The relevance of the results is primarily due to the fact that any mosquito vectors released for control are almost certainly going to be males; further, for SIT, GMM or similar approaches to be successful, the released males will have to successfully locate swarms and then mate with wild females. Thus, understanding and potentially manipulating the mating process could play a key role in future control programs. Our experience points to special challenges created by stereoscopic video of swarms. These include the expected technical difficulties of capturing usable images of mosquitoes in the field, and creating an automated tracking system to enable generation of large numbers of three dimensional tracks over many seconds of footage. Once the data are collected, visualization and application of appropriate statistical and analytic methods also are required. We discuss our recent progress on these problems, give an example of a statistical approach to quantify individual male movement in a swarm with some novel results, and suggest further studies incorporating experimental manipulation and three dimensional localization and tracking of individual mosquitoes in wild swarms.

1. Introduction

Our understanding of the mating process in swarming mosquitoes such as *Anopheles gambiae* is limited by the difficulty in quantitatively observing the mating behavior of males and females when and where it occurs naturally. Recent advances in our ability to localize and track individual swarming mosquitoes in the field via stereoscopic image analysis now enable direct observation and quantification of mating behavior (Butail et al., 2012).

Anopheline swarms usually form at dusk (though dawn swarms have also been observed; see Charlwood et al., 2002), composed almost entirely of males. They are considered mating aggregations in which males compete for access to females (Downes, 1969; Yuval, 2006). *An. gambiae* swarms often form over a "swarming marker" and nearly all mating occurs in swarms (Dao et al., 2008). Swarms change in size over the period of 10–30 min during which they occur, and have a maximum size ranging from a half dozen to thousands of males (Manoukis et al., 2009; Diabaté et al., 2011). Markers are usually an object such as a donkey cart or a contrasting area such as the opening of a well (Howell and Knols, 2009). In Mali, where we conducted our work, the sub-species of *An. gambiae* known as "molecular forms" are known to swarm over different markers: the M molecular form swarms over visually evident markers such as the ones described above, whereas the S form is almost always found over bare ground (Diabaté et al., 2009; Manoukis et al., 2009).

* Corresponding author at: USDA-ARS-PBARC, 64 Nowelo St., Hilo, HI, USA. Tel.: +1 808 932 2118.
E-mail addresses: nicholas.manoukis@ars.usda.gov (N.C. Manoukis), sb4304@nyu.edu (S. Butail), moussad@icermali.org (M. Diallo), jribeiro@niaid.nih.gov (J.M.C. Ribeiro), dpaley@umd.edu (D.A. Paley).

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Direct observation and quantification of swarming and mating has yielded insight into the fundamental biology of mating in *An. gambiae*, including information on swarm structure and a mathematical characterization of individual male movement (Manoukis et al., 2009; Butail et al., 2013). Having a measurement-based description of what occurs in nature is especially important because it enables us to form new hypotheses about mating and to test them with or without stereoscopic video analysis. Basic characterization and subsequent experiments will have important implications for any "release-based" method of vector control including Sterile Insect Technique (SIT) (Knipling, 1979), release of insects carrying a dominant lethal gene (RIDL) (Phuc et al., 2007; Thomas et al., 2000), methods involving cytoplasmic incompatibility such as via *Wolbachia* (Zabalou et al., 2004), and genetic modification of the vector such as to make it refractory to the malaria parasite (Scott et al., 2002). All of these interventions depend on the introduction of laboratory-reared mosquitoes into nature that will be able to locate the appropriate mating site, swarm with wild males and then mate with wild females, but the ability of colony-reared *Anopheles* males to perform these steps in nature is currently unknown (Boëte and Koella, 2003; Catteruccia et al., 2003; Marrelli et al., 2006). Results from other species indicate that mating behavior may be sufficiently modified in colony insects to severely limit mating with wild females (Reisen et al., 1985).

Consequently, understanding and potentially manipulating the mating process could play a key role in a variety of control programs. Our experience points to special challenges created by stereoscopic video of swarms. These include technical difficulties of capturing usable images of mosquitoes in the field and creating an automated tracking system to enable the generation of large numbers of three dimensional tracks over many seconds of footage. Once the data are collected, visualization and application of appropriate statistical and analytic methods also present special challenges. We will discuss progress on these problems, give an example of an analytical approach to describe the movement of individual males and suggest further studies incorporating experimental manipulation and three dimensional localization and tracking of individual mosquitoes in wild swarms.

2. Challenges and solutions

Stereoscopically videographing swarms ("capture"), localizing and tracking individual swarming mosquitoes, and analyzing the position and movement of these mosquitoes pose special challenges. In this section we review some problems and solutions for capture, tracking and analysis using a statistical and frequency domain based approach. For complete details on the first two challenges (capture and tracking), please refer to Butail et al. (2012).

2.1. Capture

The first and most obvious challenge to quantifying the positions and movements of individual mosquitoes in swarms is capturing the data visually. *An. gambiae* individuals are relatively small, they move at speeds of about 0.5–2 m/s, and exhibit mating behavior primarily at dusk or occasionally at dawn, when light levels are low. These conditions call for careful consideration of videographic equipment to be used.

High-definition charge-coupled device (CCD) sensors today have sufficient resolution and light sensitivity to allow imaging of individual flying mosquitoes while covering a small to medium size swarm (5–40 individuals). Our practice has been to image the swarm from a low vantage point in the direction of the setting sun, using the sky as a background. On a clear day, mosquitoes will appear as dark spots or streaks against a light background. If there are clouds, these should be kept out of the frame if possible, as they require an adaptive background-subtraction technique (Butail et al., 2012).

In order to perform stereoscopic localization, images must be acquired from two cameras simultaneously. Any deviation in the capture time will make correspondence harder (Hartley and Zisserman, 2000) and introduce error in position estimation. Our solution has been to use cameras that can be externally triggered to take an image simultaneously (i.e., an external device sends an electrical signal to the two cameras simultaneously). Most machine-vision cameras connected via a Camera Link (CL) connection can be triggered in this manner by a "frame-grabber", which also records the image pixel data to disk. A complete capture system with cameras, lenses, frame-grabber and notebook computer can be assembled for around US$10,000.

It is important to note that each camera must be "calibrated"; its optical characteristics measured in order to allow localization of objects in the field of view of both cameras. We used the MATLAB Calibration Toolbox (Bouguet, 2004) each day before filming, and found this to be relatively efficient.

2.2. Localization and tracking

For localization and tracking we apply quantitative tools from computer vision (Hartley and Zisserman, 2000; Milan et al., 2002) and estimation theory (Bar-Shalom, 1987; Cox, 1993; Veenman et al., 2001) to reconstruct three dimensional tracks of swarming mosquitoes from video footage. The tracking framework takes as input a sequence of video images and produces the estimated position and velocity of individual mosquitoes over the sequence. Complete details on the tracking algorithm are given in Butail et al. (2012), so only an overview is presented here.

Automated processing of sequential video images (Fig. 1a) extracts each mosquito's three dimensional position and velocity. The first processing step enhances each image by reducing noise and increasing contrast (Fig. 1b) and then applies a threshold over a sliding average of five nearest frames to isolate individual insects (Cavagna et al., 2008) (Fig. 1c). After this step, an insect appears in the field of view as an elongated white blob: the position of the blob in the image corresponds to the position of the insect in three dimensional space; the elongation of the blob corresponds to the component of the insect's velocity that is parallel to the image plane. We model mosquito motion as a constant-velocity Markov process with random perturbations (Bar-Shalom, 1987), which allows the tracking software to predict where in the next pair of images the blobs are expected to be seen.

Based on position and velocity of tracked mosquitoes, missing measurements in the track (if they exist) are sought by lowering the threshold in the region where the blob is expected to occur. The next step validates the position and tracking estimate generated by the epipolar constraint (the geometry of stereo vision) and then updates the mosquito position such that it is consistent with the individual motion model and minimizes the error between the predicted value and the measurement. Blobs that are expected to arise from multiple mosquito motions are split into individual streaks by fitting elongated streak shapes onto the pixels until the blob is completely covered. Our automated process to address the matching problem is not guaranteed to work at all times due to occlusions between mosquitoes that are yet undetected, or fading out of view, so the tracks are later verified by a human analyst. The complete, supervised tracking system is relatively fast; an experienced operator can track an individual mosquito for 60 s in under 30 min of work.