Lights, Camera, Flight: Refining the negative phonotaxis characterization in *Gryllus bimaculatus* using markerless pose estimation software DeepLabCut

Ryan M. Kang, 2025

When crickets fly at night, the insect must be able to recognize the high-frequency tones that their nocturnal predators, bats, emit. After detection, crickets must turn away and avoid being preyed upon. To study the evasive locomotive behavior elicited upon recognition of the predatory signal (negative phonotaxis), experiments have included throwing up the cricket to induce flight or attaching them to a stick by their pronotum in front of a fan (Lorenz, 2007; Zeng et al., 2014; Sun et al., 2020; Ren et al., 2022). However, deciding precisely how evasive flying behavior should be characterized and analyzed while keeping the organism alive remains foggy. Previous research found that cricket flight positions were similar to those in locusts, but characterizing what 'flying posture' the cricket must be in first before being able to be used in analysis remains a mostly qualitative indicator that likely differed between studies and researchers (Moiseff et al., 1978). Typically, flight posture is identified as a stiffened abdomen is stiffened, outstretched hind legs, and tucked forelimbs (Moiseff et al., 1978). Previous studies have used strict researcher-based scoring systems subject to more bias or exclusion of data (Moiseff et al., 1978; Tomioka & Yamaguchi, 1980). However, advancements in machine learning and markerless pose estimation have been a great tool in addressing animal behavior research to produce statistically robust quantitative data (Westwood et al., 2023; Hayakawa et al., 2024; Kyalo et al., 2024).

Researchers investigating cricket behavior have used DeepLabCut, a markerless pose estimation software that accelerates posture analysis by digitally attaching points onto cricket body parts on film then having a neural network go through thousands of frames to analyze whole videos at once (Mathis et al., 2018; Hayakawa et al., 2024). Previous research conducted in the Horch lab investigating behavioral neuroplasticity have used DeepLabCut to assess how accurately crickets can react to predatory calls after injury (Scholes, 2020; Small, 2023). Nevertheless, limitations of video quality, small number of labeled body parts, and an unestablished data workflow have muddled potential results (Small, 2023). Therefore, my project this summer has been to fully recharacterize a quantitative measurement of flight posture by refining the software pipeline for analysis, hardware improvements to increase video quality for lower error, and more digital tracking points onto the cricket for more reference points to analyze (Figure 1).

By flying crickets and recording them with an updated camcorder, we improve current recording setups currently established by Hayakawa and colleagues (2024), increasing the number of frames captured from 1frame/min to 59.95 frames/sec and increasing the number of tracking points from six to 21 points. As improvements in recording increased the quality and quantity of collected data, we are currently analyzing various reference points to see if we can isolate the exact snapshot of flying posture and a quantitative scale to characterize how well each cricket flies (Figure 2). The lab is currently working in conjunction with the math department to establish ranges of acceptable variation, measures of posture based on 2D coordinates, and multi-dimensional analyses for a reduction in data complexity.

Preliminary results suggest that a cricket will have a noisier flight if flown repeatedly, but may respond better overall to predatory bat noises. Data regarding double-stranded RNA injection experiments have been recollected for corroboration with the new pipeline but have not yet been analyzed. This summer project will be continued through the school year as my honors project.

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Figures:

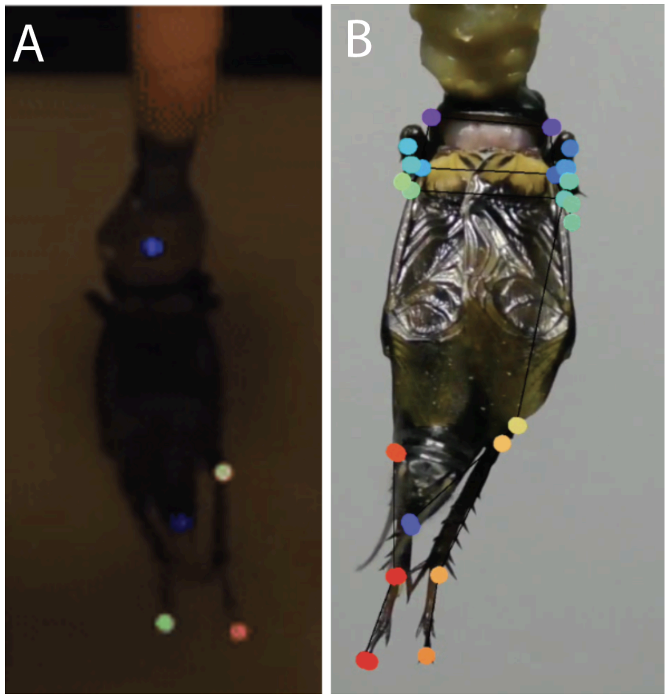

Figure 1. A comparison of labeled cricket videos according to time experiment was conducted. A) An example of an experimental cricket's tethered flight conducted in 2023. There are six tracking points (one not shown), including the anchor point. B) An example of an experimental cricket's tethered flight conducted in Summer 2024. There are 21 tracking points (3 not shown), with a revised tracking anchor point attached to the cricket's pronotum and wing hinge. There is better lighting and improved resolution.

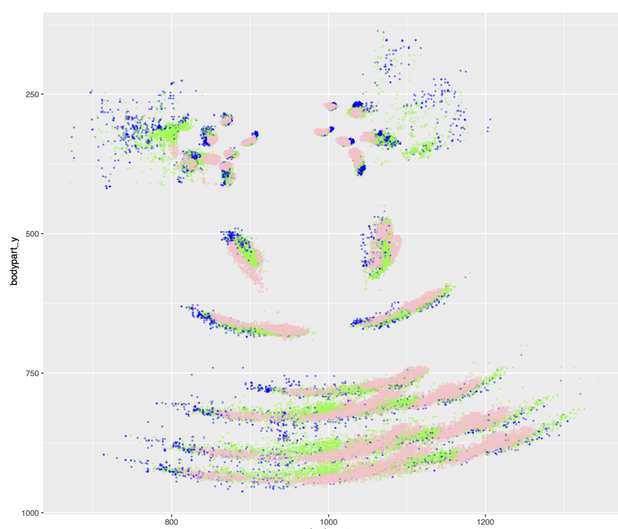

Figure 2. Labeled tracking points of one cricket on a 2D coordinate plane. Each color corresponds to labeled body parts plotted all at once to identify potential standard flight postures (pink for in-flight, green for transition state, blue for out-of-flying position). The data shown here is not finalized yet.

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