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Male Beetle Courtship Dance Analysis by DeepLabCut Technology

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HONR 499
Senior Thesis/Capstone
Mentor: Doug Emlen

Male Beetle Courtship Dance Analysis by DeepLabCut Technology

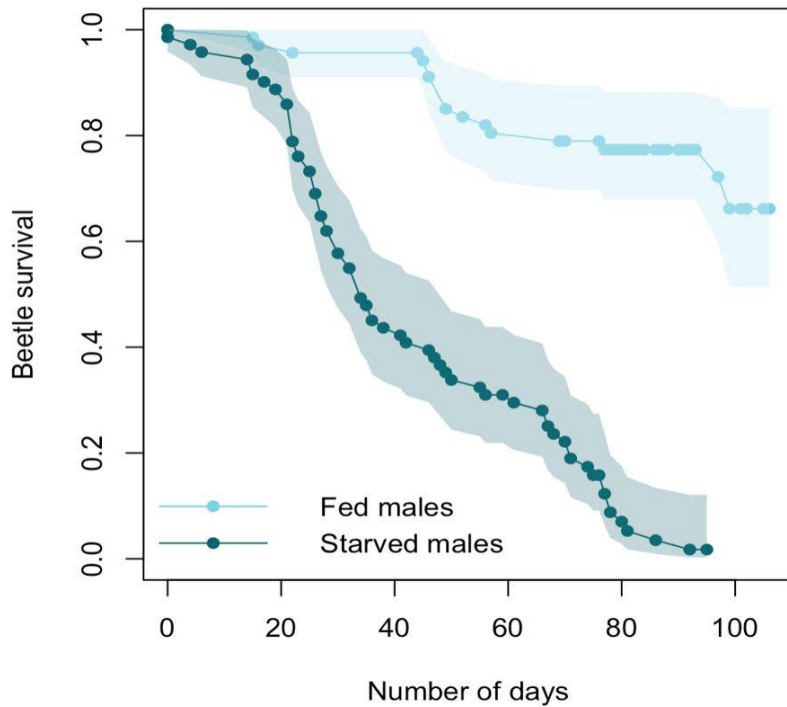
In many species, males compete for access to females. This often occurs through elaborate displays of courtship songs, dances, large plumes, or flashes of brightly-colored feathers or fur. In these species, the males compete to display the most attractive courtship alongside one another and the females actively choose her mate amongst these males. In other species, males compete directly with rival males. These species are characterized by large body sizes and weapons that attribute the male having a higher fighting success. In heavily-weaponed species, males sort amongst themselves and the winning males thus have higher mating opportunity. It was anticipated that in these species, females aren't actively making a choice regarding her mate. This passive behavior by the female assumes that the winning male would also be the best mate with good genetics and nutrition for her offspring. Recently, there have been a few exceptions to the heavily-weaponed species pattern, including the Japanese Rhinoceros beetle.

The Japanese Rhinoceros beetle are characterized by long pitch-fork like horns and the male-male fights over feeding territories and females. Larger males with bigger, longer horns win their fights and thus win access to females. We expected that the Rhinoceros beetle female followed in suit with other heavily-weaponed species and practiced little or no active mate choice. However, in this species, females have been seen to routinely reject males.

After the males win their battles against rival males, they must perform an elaborate courtship for the female before mating. The courtship is comprised of stridulatory songs, trembling, and oscillating dances. Many animal and insect songs has been studied in species like crickets and grasshoppers. The Emlen lab has identified at least two unique songs that the Japanese Rhinoceros male beetle produces. There is ongoing research into analyzing the specifics of the songs which may include patterns, a third song type, and volume. The trembling and dance aspect of the courtship has been studied less than the song aspect. This trend is true across many species. The Emlen lab expected that after the hours of repetitive battles, the male

beetles would be exhausted after having depleted their energy reserves. This thought process presented the idea that females select their mates by measuring the male's short-term body condition. The Japanese Rhinoceros beetle females only mate once in their lifetime. Females thus care about the amount of nutrients they receive to help support the offspring (spermatophore) and the strength of the genetics from the male.

To test the idea that trembling dances signal short-term body condition to the female, the



lab first had to manipulate diet. Back in the summer of 2021, hundreds of beetles underwent a diet comparison study. A restricted diet resulted in the stressed, or starved beetles, to die sooner compared to the fed beetles (Figure 1).

Figure 1: Starved males died off sooner than males fed *ad libitum*.

Body size was also uncoupled from condition through this study. Starved, or stressed, males weighed less for their size than fed males (Figure 2). Size was determined off a width and length body comparison. The Japanese Rhinoceros beetles are a metamorphic animal that undergo a 10-month larval period before molting into a pupa and then into an adult. This beetle's body size is fixed for their lifetime.

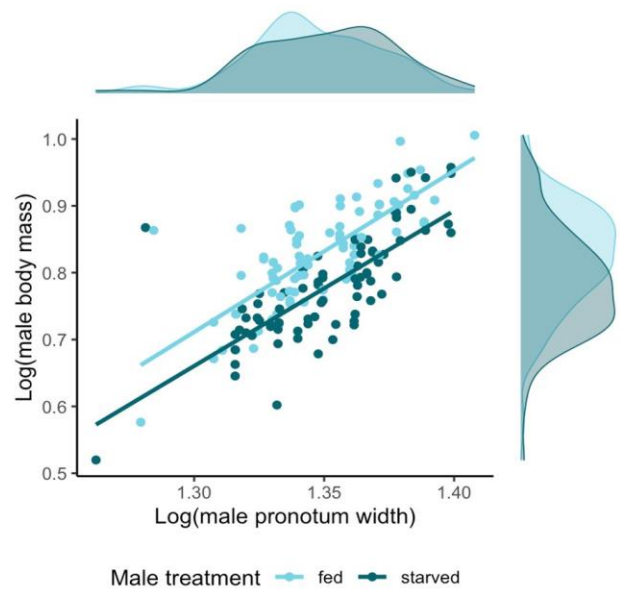


Figure 2: Starved males weighed less for a given size than males fed *ad libitum*.

Out of the hundreds of courtships that were studied and analyzed from the summer of 2021, 70 courtships' dances were analyzed. 35 courtships involved a fed male and 35 courtships involved a stressed male. The dances were analyzed using DeepLabCut, an estimation algorithm technology that is commonly used in tracking animal behavior. DeepLabCut tracked the movement of the beetles bodies during courtship. The displacement of the male's body was tracked with unique landmarks (Figure 3). The confidence of the program then further allowed the speed of the back-and-forth, or oscillating, movements to be calculated.

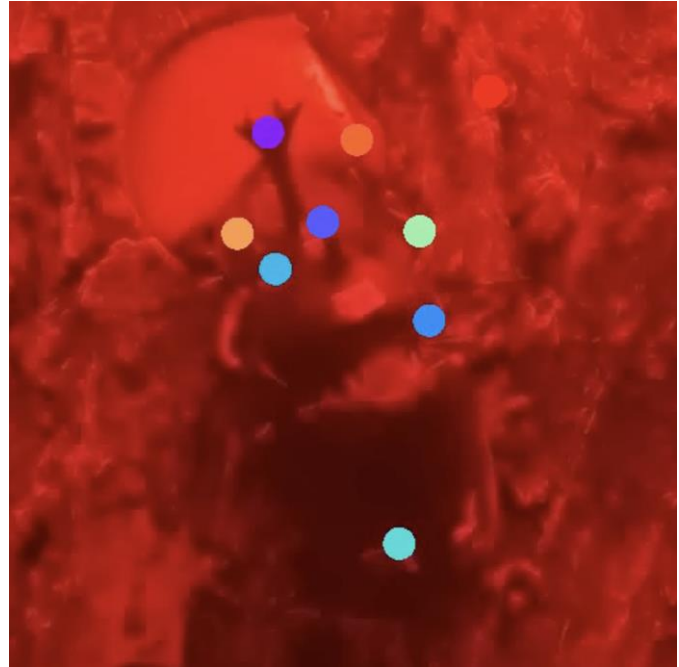


Figure 3: A labeled frame: markers placed on both the male's and female's bodies .

The results of this study confirmed the idea that stressed male beetles do not have the same energy reserve that a fed male beetle does. Thus the stressed

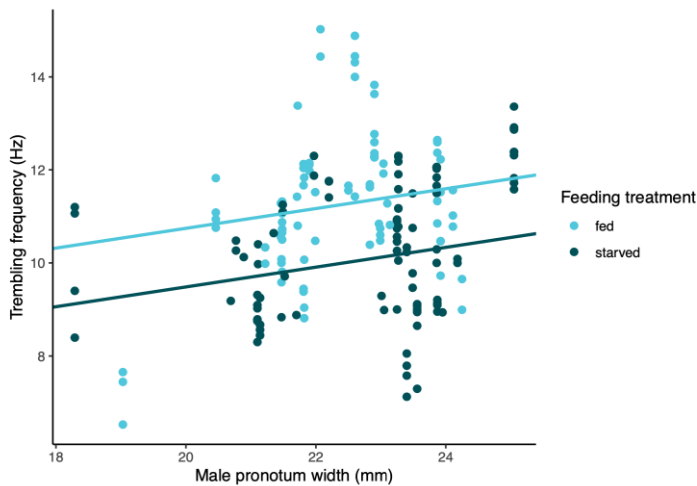


Figure 4: Male size versus tremble rate.

beetle gets tired quicker and trembles slower (Figures 4 and 5). While body size had a minor role in tremble rate, tremble rate was found to be more dependent on short-term condition (Figure 4).

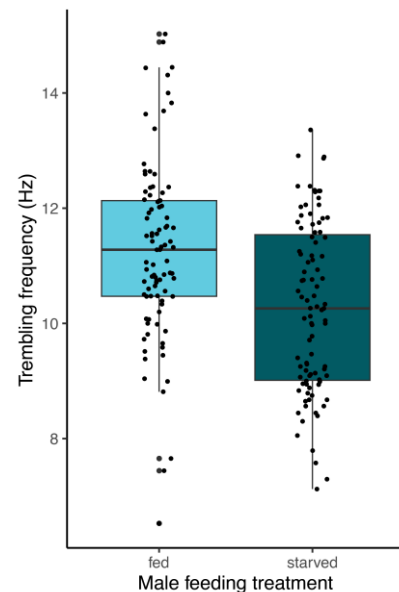


Figure 5: Feeding treatment versus tremble rate.

This preliminary study of Japanese Rhinoceros beetles utilizing DeepLabCut exemplified the complexity of female choice. This species does not follow the trend of heavily-armed species and the females actively make mate choices. We found that the male's trembling dances does signal an aspect of his short term condition. The next steps for the Emlen lab include looking into related beetle species and seeing what similarities are common between courtships.