Nocturnal butterflies and bats: a landscape-genomic approach to characterizing a landscape of fear

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Predator-Induced Stress

Predator-induced responses in prey organisms have, until recently, been viewed as acute and transitory, with minimal lasting impact on population demographics. Recently, however, studies have confirmed that indirect and direct interactions with predators induce stress responses in prey that are similar to chronic stress in humans, conferring significant impacts on fitness, activity, and survival.

Within the insect brain, predator-induced stress responses are likely associated with the up-regulation of certain genes that produce peptides, biogenic amines, and hormones that aid in adaptive responses to predator exposure, such as energy mobilization and increased alertness. Animals which cue in on predator signals to avoid predation may have chronic stress responses that vary across a landscape heterogeneously according to predator presence and activity.

Figure 1. Phylogeny demonstrating the proposed relationship between Hedylidae, its sister butterflies, and other moth families

Figure 2. *Macrosoma heliconiaria* captured near Fort Sherman canopy crane station in Colón, Panama

Macrosoma heliconiaria (Lepidoptera: Hedylidae), one of 42 species of American butterfly moths, are equipped with tympanal ears at the base of their forewings (Fig. 1, 2, 3). These ears, thought to be homologous with the nymphalid (Lepidoptera: Papilionoidea) Vogel's organ, are tuned to high-frequency ultra-sound produced by insectivorous aerial-foraging bats. In reaction to recorded bat calls, flying *M. heliconiaria* speed up and change direction erratically in a defensive flight maneuver (Fig. 4).

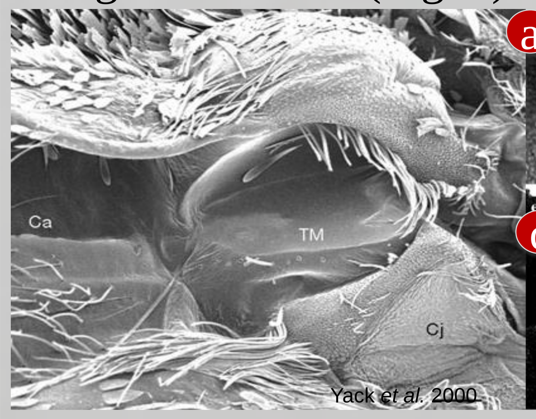

Figure 3. Ear of *M. heliconiaria* located ventrally at the base of the forewing

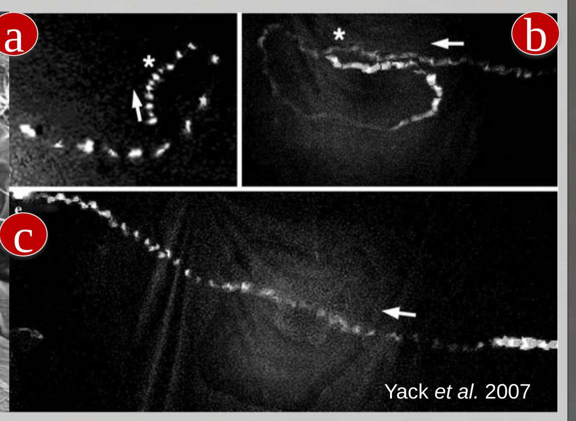

Figure 4. a & b. Example of the evasive flight maneuver of *M. heliconiaria* upon exposure to ultrasound; c. example of normal, non-evasive flight

Neurochemistry & Genomics

These flight maneuvers likely represent metabolic and behavioral phenomena which require the quick up-regulation of genes that code for the production of biogenic amines, neurotransmitters, and other peptides that modulate effective predator avoidance pathways (Table 1; Fig. 5).

Table 1. Chemicals and their roles in the insect stress response

chemical	abbrev.	family	function
octopamine	OA	biogenic amine	neurotransmitter, neurohormone; affects arousal, increases heart rate, modulates muscle activity
dopamine	DA	biogenic amine	neurotransmitter; affects arousal
adipokinetic hormone	AKH	metabolic hormone	increases fat metabolism; allows increased energy use
diuretic hormone	DH	metabolic hormone	initiates diuresis induced by crop draining into hindgut after energy mobilization
heat shock proteins	HSP70	chaperone	protects cells from oxidative stress and excess protein misfolding
cortico releasing hormone-binding protein	CRH-BP	chaperone	modulates hormonal release

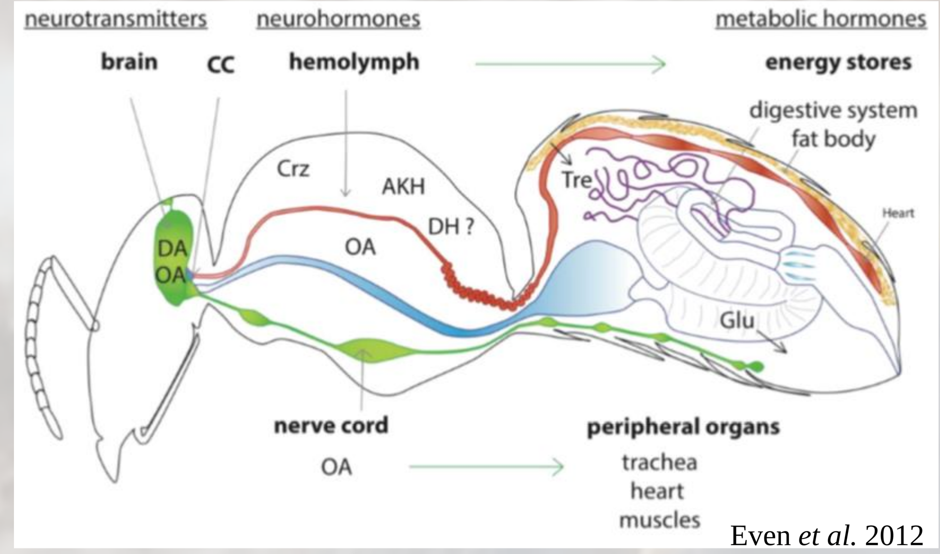

Figure 5. Diagram of the general insect stress response

Using HPLC, I will measure the concentrations of these chemicals in the brain vs. nerves innervating the ear to pinpoint the response.

Simultaneously, I will build a *de novo* genome for *M. heliconiaria* so that reliable transcriptomic assays can be carried out. Two groups of adults reared from egg will then be differentially exposed to recorded bat calls and a transcriptomic sweep for gene expression patterns related to stress will be completed.

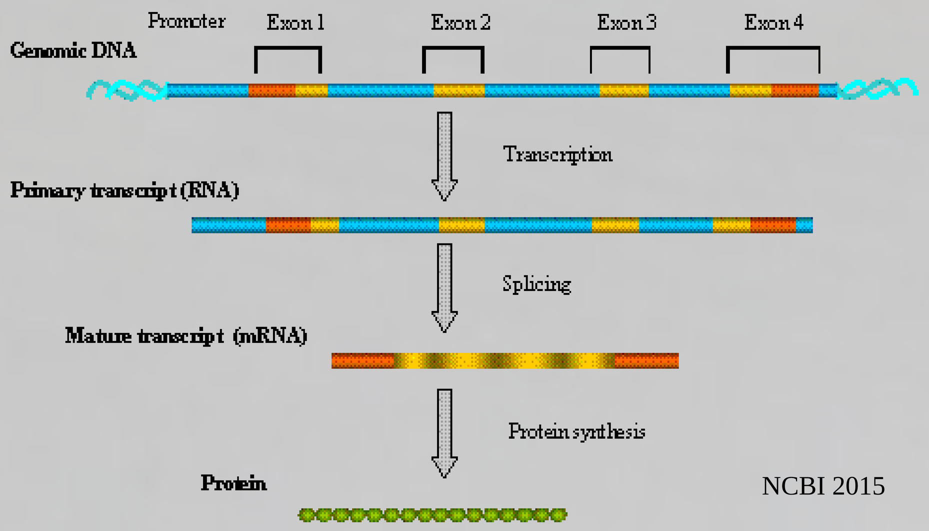

Figure 6. Diagram explaining how genes are expressed from DNA to form functional proteins

The Landscape of Fear

By integrating the genome, gene expression patterns induced by predator exposure, and estimates of *M. heliconiaria* abundance, I will map indirect predator stress along several landscape-scale transects situated perpendicular to riparian zones, ranging from areas of high insectivorous bat predation (riparian) to low predation (interior forest).

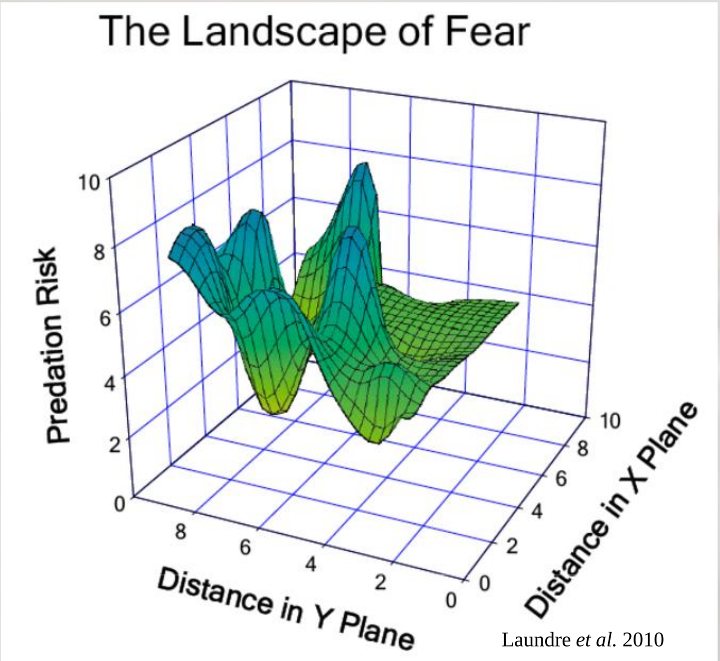

Figure 7. Visual representation of a landscape of fear, with the physical coordinates of a landscape on the x, y-planes and predation risk, or predation response, on the z-axis

Figure 8. Example of a bat-moth predation event, the dynamics of which may create a landscape of fear

Materials and Methods

- Collection of butterflies and bat recordings will be from Gamboa, Fort Sherman, and Barro Colorado Island, Panama.
- A Qiagen MagAttract HMW DNA Extraction kit was used to extract DNA
- DNA degradation has been assessed via agarose gel electrophoresis (Fig. 9)
- DNA will then be sent to the Biotechnology Center at UIUC for sequencing
- 2 x 250 bp mate-pair libraries will be constructed based on 3, 8, and 15 kb length sequences.
- The DISCOVAR *de novo* genome assembler (ALLPATHS-LG) will then be used to compile the genome and annotation will commence.
- Conduct transcriptomic analyses on wild individuals while also monitoring bat call frequency as a proxy for predation risk
- Map bat call frequency, stress, and butterfly abundance across a landscape using ArcGIS

Current Results

Two *M. heliconiaria* individuals have been found thus far and two DNA extractions have been successfully completed at the Naos labs (260/280 A = 1.55 and 1.63; concentrations = 25.7 and 20.7 ug/mL, respectively).

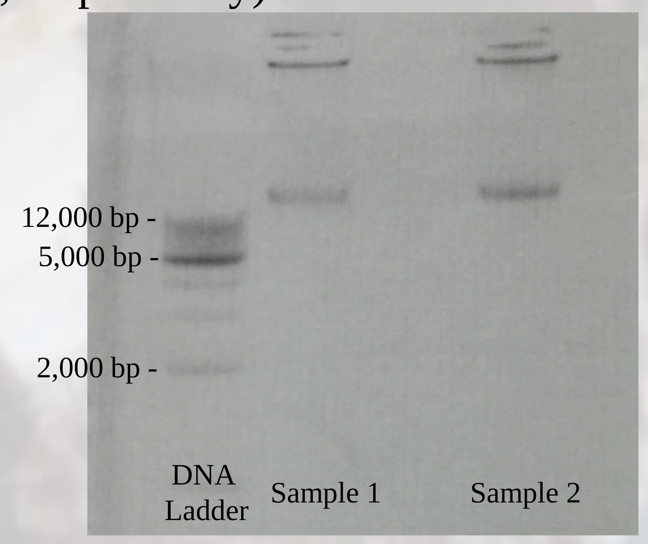

Figure 8. DNA ladder showing the length and quality of the *M. macrosoma* HMW DNA extracts

Conclusions

This project will produce insights on

- **The divergence of butterflies from moths**
- **The processing of predator cues within prey individuals**
- **The gene expression changes that occur in the face of a predator**
- **The use of the "Landscape of Fear" framework for explaining indirect predator influences on prey dynamics**

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