Exploring the Role of Notch Cell Signaling in *Harpegnathos saltator*

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Abstract— **Notch cell signaling is a well-known and an important cell-signaling pathway, responsible for processes including differentiation and cell fate specification as well as linked to multiple diseases. This study attempts to increase knowledge of eusocial insect colony structure and determine the effect of Notch signaling on reproductive behavior of *Harpegnathos saltator* workers by inhibiting the Notch pathway with DAPT. Results from this experiment indicate that inhibition of Notch is correlated with reproductive development in *H. saltator*. This correlation and the mechanisms behind it will facilitate the study of other eusocial insects. In addition, the association of the Notch pathway with diseases like cancer may yield important information regarding the role of Notch in animals. Future research should focus on exploring eusociality in *H. saltator* and expanding upon the understanding of Notch.**

I. BACKGROUND

Like other eusocial insects, the *Harpegnathos saltator* ant colony consists of a reproductive queen and nonreproductive workers. However, in the absence of a natural queen, *H. saltator* workers undergo a unique transition, activating their ovaries and becoming gamergates, or pseudo-queens. The Notch cell signaling pathway was discovered in the wing of Drosophila, and is involved in differentiation, proliferation, and apoptosis in multiple organisms. The pathway's importance is corroborated by links drawn to various diseases, primarily cancer [2]. Specifically, Notch is linked to numerous tumors, from neoplasms to melanomas to carcinomas [3]. As a result, the study of Notch is a subject of interest to many researchers. A recent study has demonstrated that inhibition of Notch overcame the repressive effect of queen honey bees, causing the workers to become reproductive [1]. This raises the question of how inhibition of the Notch cell signaling pathway affects reproductive activity in *H. saltator* workers.

II. METHODS

The experiment was conducted with a *H. saltator* colony of twenty-five individuals from the same origin colony. The colony contained three sections: the source, experimental, and control group. Both the experimental and control group contained five age-matched callow workers. Experimental individuals are denoted by a two-letter name beginning with G, while control individuals by a two-letter name beginning with Y. The second letter of the name is determined by the color of each individual ant. DAPT (N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester) was dissolved in a dimethyl sulfide (DMSO) solvent and introduced into the food and water source of the colony to inhibit the signaling pathway. Colony air was kept arid and ants were only provided treated water and crickets to ensure exposure to treatment. Control and treated ant ovaries were fixed, stained with DAPI and phalloidin, and mounted to produce confocal images. Finally, a qPCR was run with Notch gene *bHLH2* and reference gene *GAPDH* to determine the fold change in *bHLH2* expression [4].

	Fold change compared to control individuals	
Treated individual	Control individual YG	Control individual YY
GG	1.141-fold decrease	1.181-fold increase
GW	1.227-fold decrease	1.117-fold increase
GO	1.385-fold decrease	1.028-fold decrease
GY	2.497-fold decrease	1.853-fold decrease

Figure 1: *Comparison of fold change in bHLH2 due to treatment. Differences between C_T values of bHLH2 and GAPDH are compared in treated and control individuals to determine the fold change due to treatment in bHLH2 expression [4]. Fold changes largely display a decrease in bHLH2 expression.*

III. RESULTS AND DISCUSSION

The majority of treated and control ants had slight development, with either 1 or 2 egg chambers visible. However, control ants YG and YY had 2 and 4 egg chambers visible, respectively. That treated individuals GG and GW show a decrease in expression compared to YG, but an increase in expression compared to YY, demonstrates the negative correlation between *bHLH2* expression and ovarian development. Furthermore, treated ant GY had 4 egg chambers visible, further establishing this correlation.

This experiment has several implications. For one, demonstrating that Notch inhibition triggers reproductive development introduces a valuable method of starting oogenesis in *H. saltator* and facilitating future research on gamergates. Furthermore, understanding the effect of Notch on *H. saltator* provides insight into the role of Notch on a molecular level. Clarifying Notch's biological role in this way enhances our knowledge of Notch in diseases like cancer. Finally, studying Notch in the gamergate transition allows for insight into *H. saltator* colony structure and eusociality as a whole. With this in mind, future research should focus on determining the molecular mechanisms behind Notch inhibition.

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V. REFERENCES

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