

# Nutritional Influences on Sexual Selection in Butterflies

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## Introduction

- Reproduction is an energy intensive and metabolically costly portion of any organism's life
- Males insert sperm and nutrient filled sacs (spermatophores) into the female reproductive tract (bursa copulatrix) during mating
- Spermatophores are digested over time, sometimes with the help of signum<sup>1</sup>
- Nutrients like nitrogen, sodium, and phosphorus, influence wing coloration and ornamentation<sup>2</sup>
- Anthropogenic activities like agricultural fertilizer use and burning fossil fuels have impacted biogeochemical cycling and have lead to increased availability of some previously scarce nutrients<sup>3</sup>
- Diet nutritional content of different butterfly species can be determined by evaluating the concentration of key minerals within the host plant
- With less nutrient availability from plants, there is a stronger pressure on females to fully digest the spermatophores to maximize their metabolic benefits<sup>4</sup>

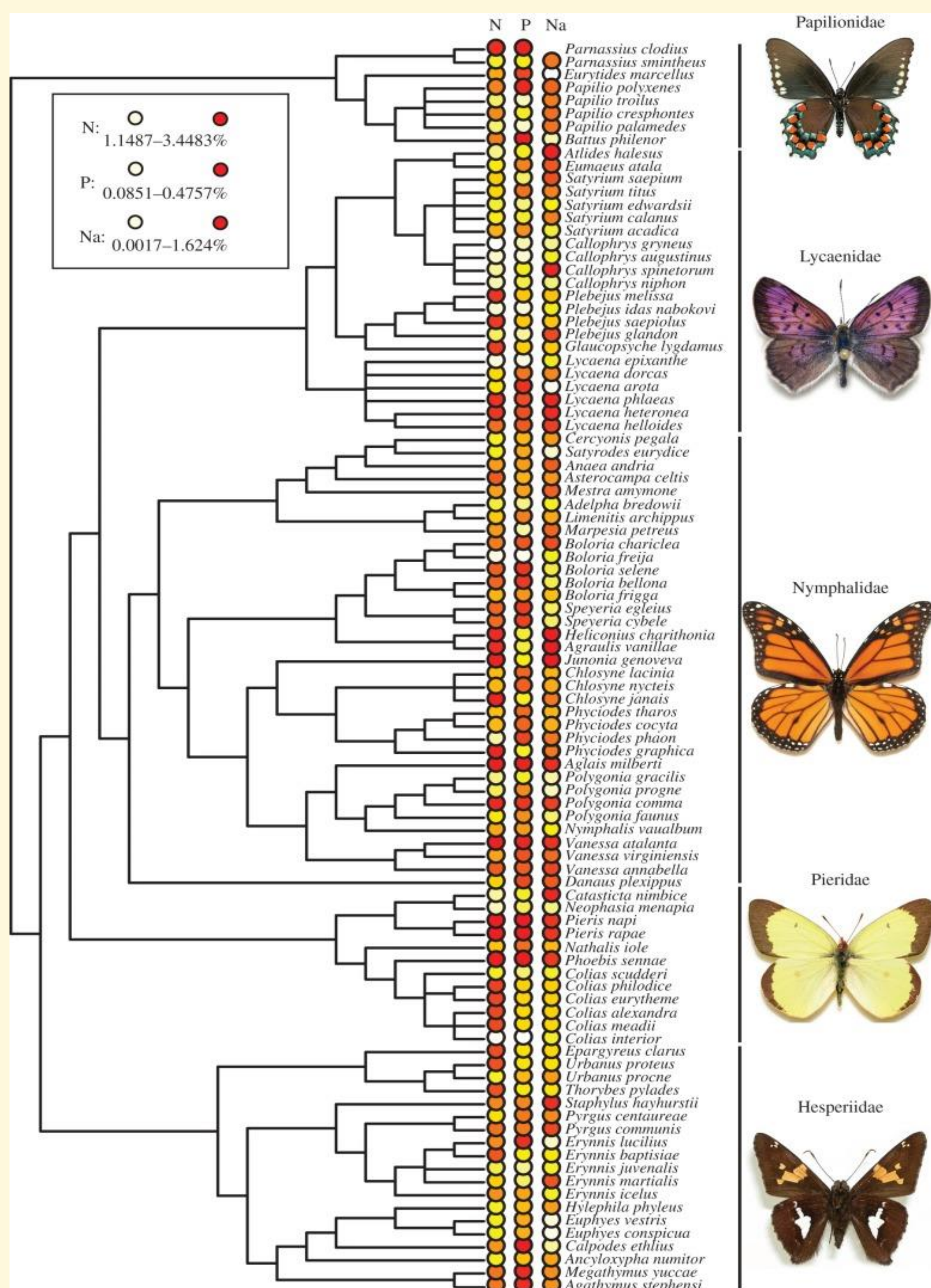
## Hypothesis

Females with access to more nutritious host plants will have a smaller total signum area as they will not be as environmentally pressured to extract all the nutrients from spermatophores to survive and reproduce.

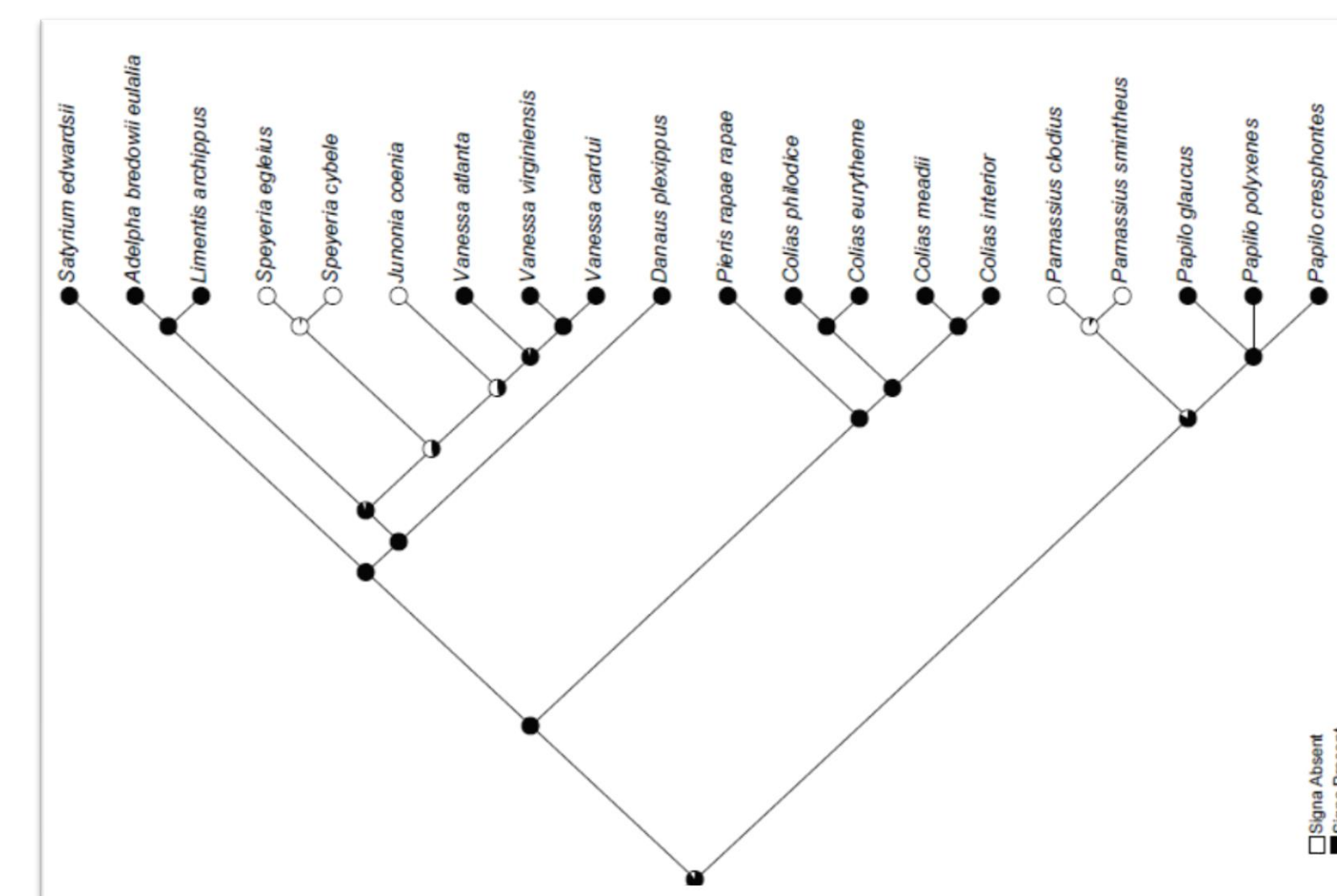
## Methods

- Sampling was done on 53 species of butterflies with varying host plant nutrition; butterfly host plant information was obtained from Scott *et al*<sup>5</sup>
- Nitrogen and phosphorus content of host plants was obtained from Borer and sodium content was obtained from Watanabe *et al*<sup>6,7</sup>
- Butterfly abdomens were dissected and preserved in 1x phosphate-buffered saline (PBS) buffer
- Abdomens were laid flat and sent for further bursa copulatrix dissection and imaging of signum
- Bursa copulatrix morphology was analyzed in ImageJ by examining left and right lobe height, and signum presence, quantity, and area
- Teeth (signa/signum) where defined to be any pointed structure that was more melanized than the surrounding bursa copulatrix area

## Results

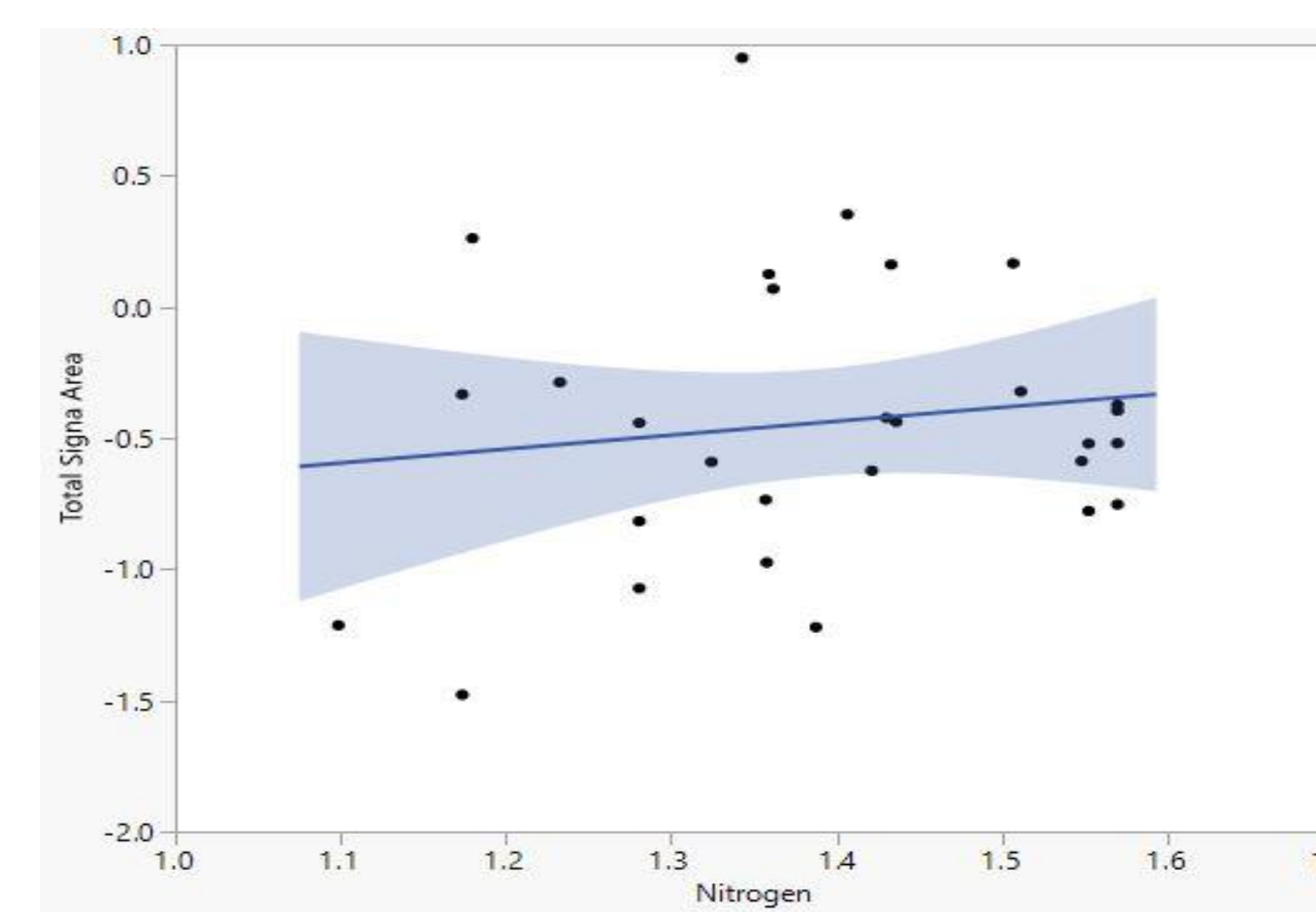


**Figure 1:** Phylogeny of butterfly species and host plant nutrition levels.<sup>4</sup> Lower (white) and higher (red) concentrations of nitrogen, phosphorus, and sodium are represented with the colored circles. The 53 species in this study were obtained from this phylogeny.

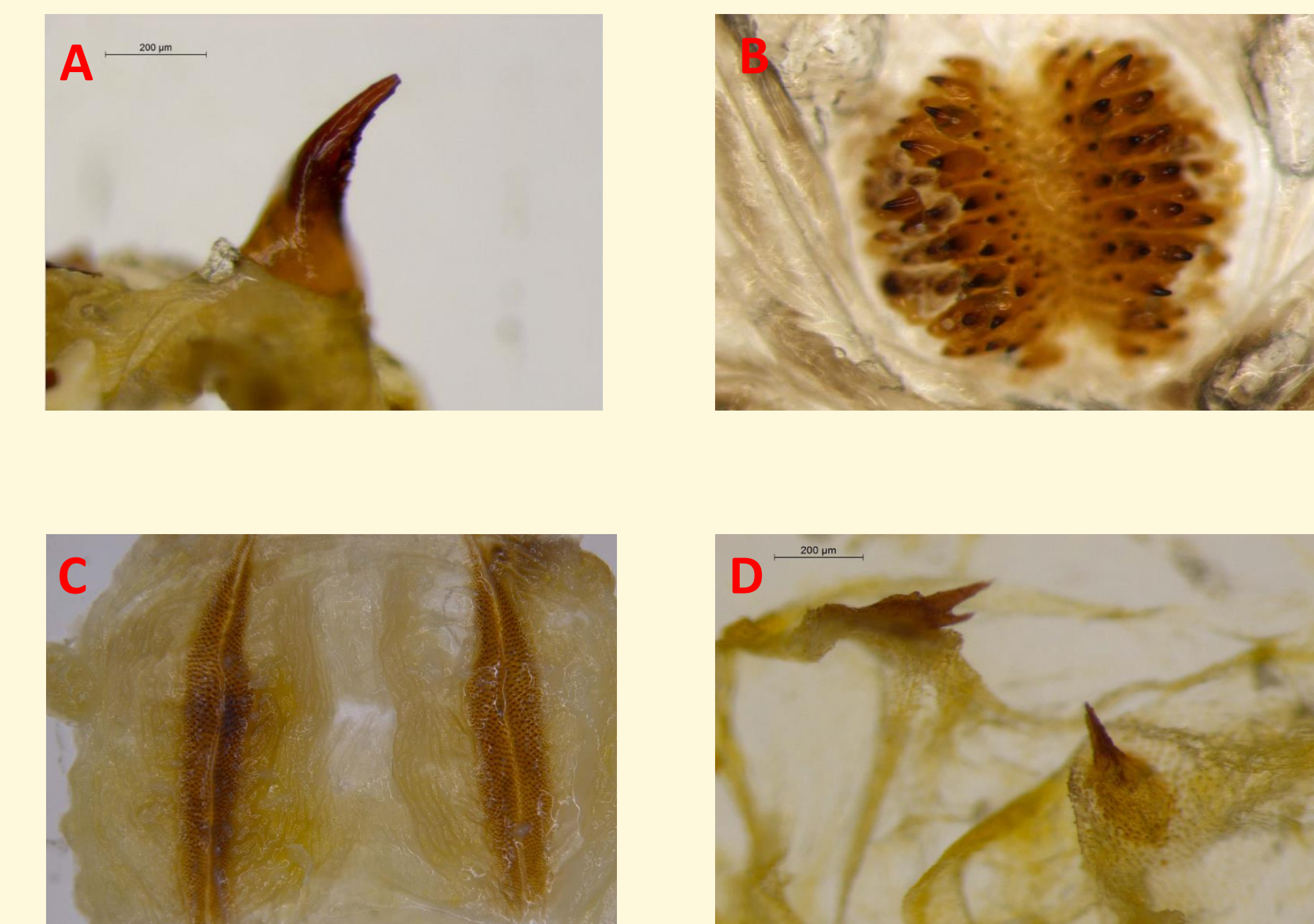


**Figure 2:** Phylogenetic tree of the presence or absence of signa in the bursa copulatrix of 20 butterfly species. The presence of signum is tied to polyandrous species.

- The presence of signum is associated with polyandrous butterfly species, as they need to efficiently breakdown spermatophores before future mating occurrences
- Species found with no signum were predominantly monogamous
- No significant relationship was found between nutrition and total signa area, but there was a slight positive correlation between nitrogen content of the host plant and signum area
- No correlations were found between total signa area and host plant phosphorus and sodium concentrations



**Figure 3:** Total signa area (µm<sup>2</sup>) vs. nitrogen concentration of host plant (mg N/g dried plant). A one-way ANOVA [F(1,27)= 0.53, p=0.475] showed insignificant differences between host plant nitrogen content and total signa area.



**Figure 4:** Signum A) single piercing signa B) single, bi-lobed signum C) paired and elongated signum D) paired and piercing signum

## Conclusions

- There were no significant relationships between total signum area and host plant nitrogen, phosphorus, or sodium concentrations
- Nutrition may affect mating behavior in butterfly species, but it was not quantitatively identified in relation to signum area in this study [F(1,27)= 0.53, p=0.475]
- Females that receive larger spermatophores with more nutrients have a longer time between mating occurrences<sup>8</sup>
- Future research should investigate the relationship between nutrition, signum area, and varying mating systems (monogamy vs. polyandry)

## Acknowledgements

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