

# Plasmodium falciparum oocyst developmental transcriptomes and responses to dynamic nutrient stresses in Anopheles gambiae

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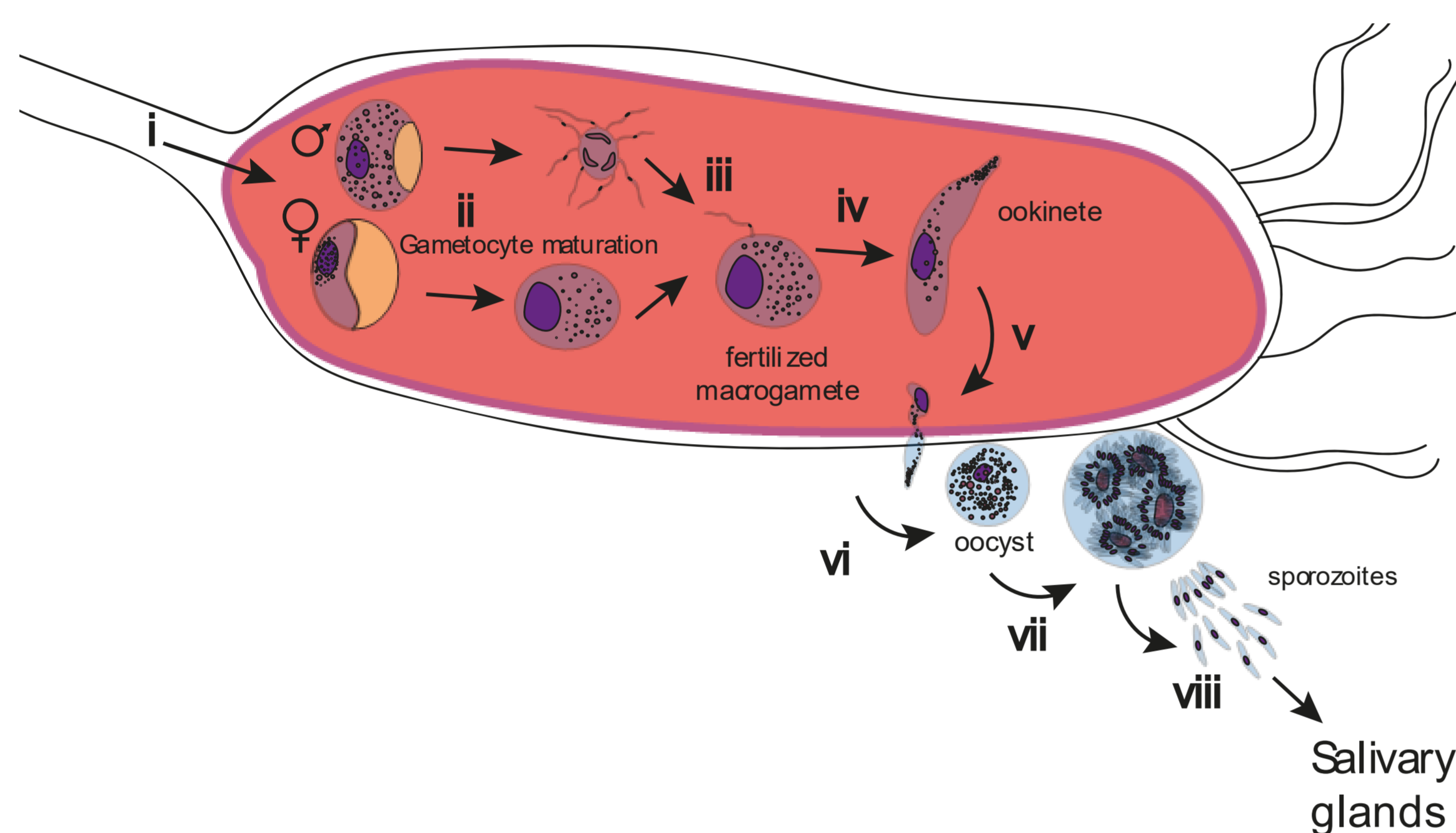
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## Abstract

Malaria remains a substantial global health burden, with a sharp rise in cases and deaths in 2020 underlining a need for novel interventions. At the oocyst stage of parasite development in the mosquito, the parasite population approaches a bottleneck and is particularly vulnerable. The oocyst is therefore an attractive intervention target, but many unknowns still surround its interactions with the mosquito. Here, we present RNA sequencing data shedding light on how oocysts respond to varying nutrient abundance and stress levels within the mosquito. We uncover a trajectory of oocyst development with few differences between early oocysts from drastically different mosquito environments, whilst the switch from oocyst growth to differentiation occurs at different rates depending on the mosquito nutrient levels. The profile of AP2 transcription factors implicated in these two programs is described.

## Background

With about 627,000 deaths and 241 million cases of malaria in 2020 and a substantial rise from previous years fuelled by emerging parasite and mosquito resistance to interventions, the need for new antimalarial strategies is critical<sup>1</sup>.



In the mosquito, the parasite population reaches its lowest level at the oocyst stage which is responsible for producing thousands of infectious sporozoites. Oocysts rely on the mosquito for nutrients, siphoning carbohydrates and lipids circulating in the haemolymph to fuel growth and differentiation<sup>2</sup>. The importance of mosquito bloodmeals in aiding oocyst development has been revealed<sup>3</sup>. At the molecular level, how the oocysts respond to such dynamic nutrient conditions, however, remains unknown.

## Aims and Significance

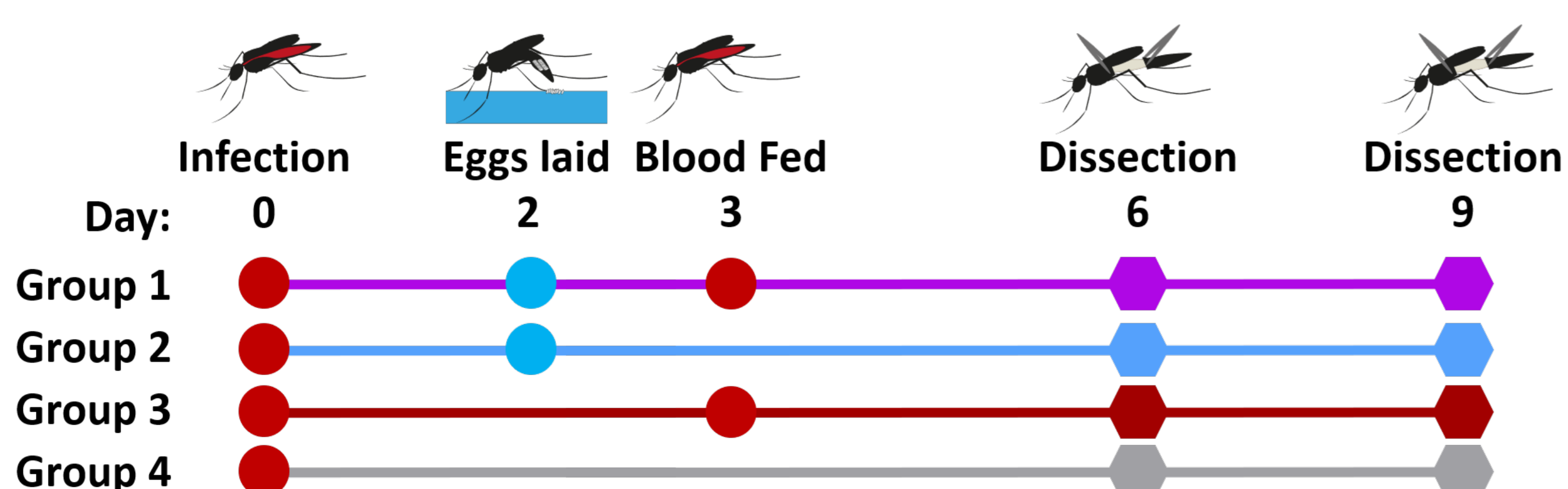
This work aims to characterise the transcriptomic response of human malaria parasite *P. falciparum* to dynamic nutrient conditions in the mosquito by:

- Investigating the parasite response to mosquito bloodmeal-induced nutrient depletion and nutrient boosting,
- Characterising changes to the oocyst transcriptome across time as the parasite switches from growth to sporozoite production, and
- Identifying transcription programmes directing oocyst development.

Understanding oocyst responses to natural mosquito behaviours will help to identify new intervention targets and enhance our understanding of malaria transmission biology.

## Methodology

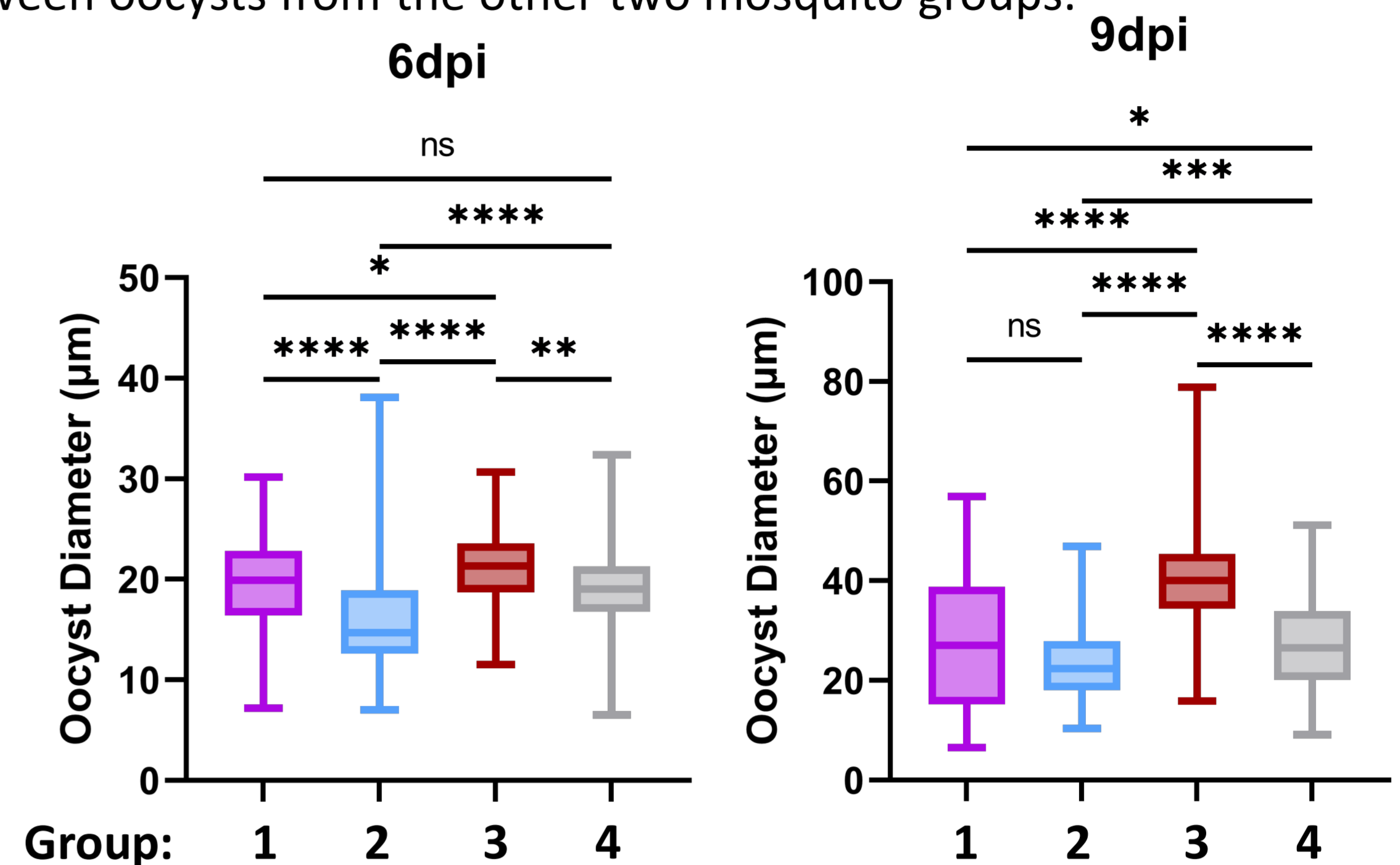
Four experimental conditions (groups) were established from infected mosquitoes that had a supplementary bloodmeal and/or were allowed to egg lay. At 6 and 9 days post-infection (dpi), oocyst sizes were determined and RNA samples were collected for sequencing.



## Results

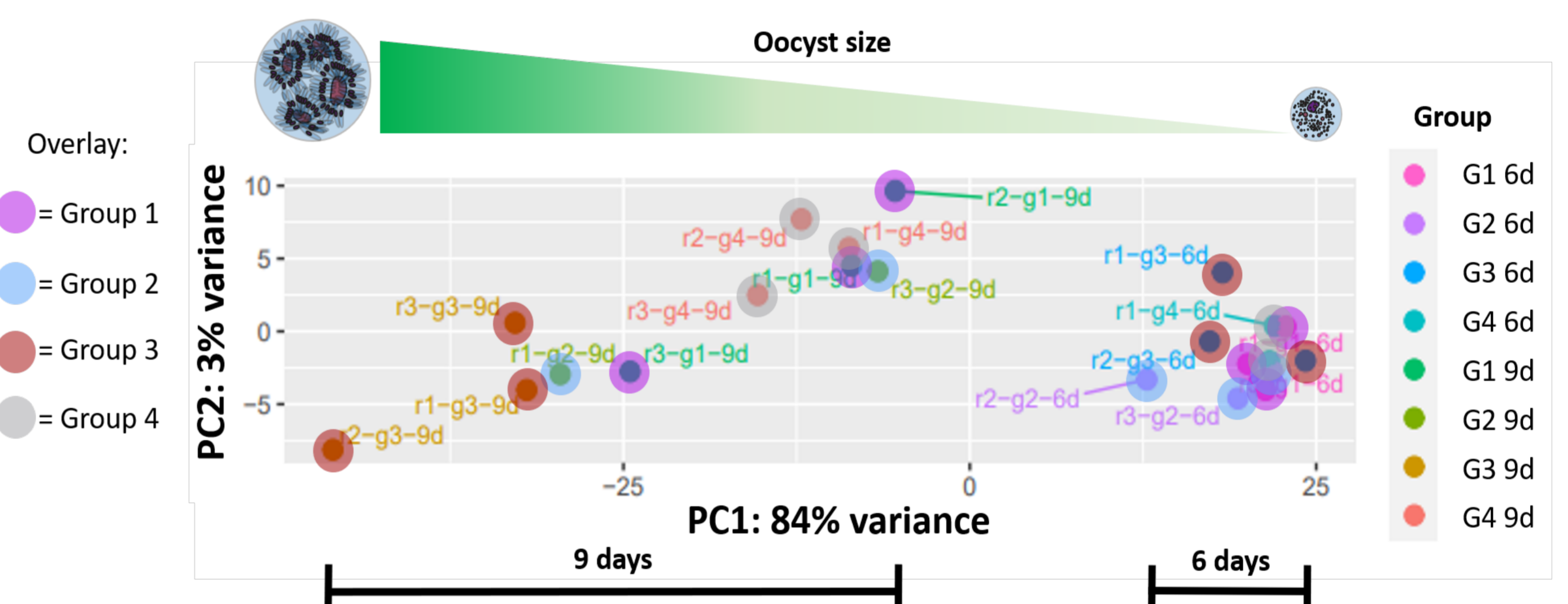
### 1. Nutrient level impacts oocyst growth

Oocysts from blood-fed only mosquitoes (Group 3) are larger at both 6 and 9 days than other groups, whilst those from mosquitoes that only laid eggs (Group 2) are smaller than other groups. No major differences are seen between oocysts from the other two mosquito groups.



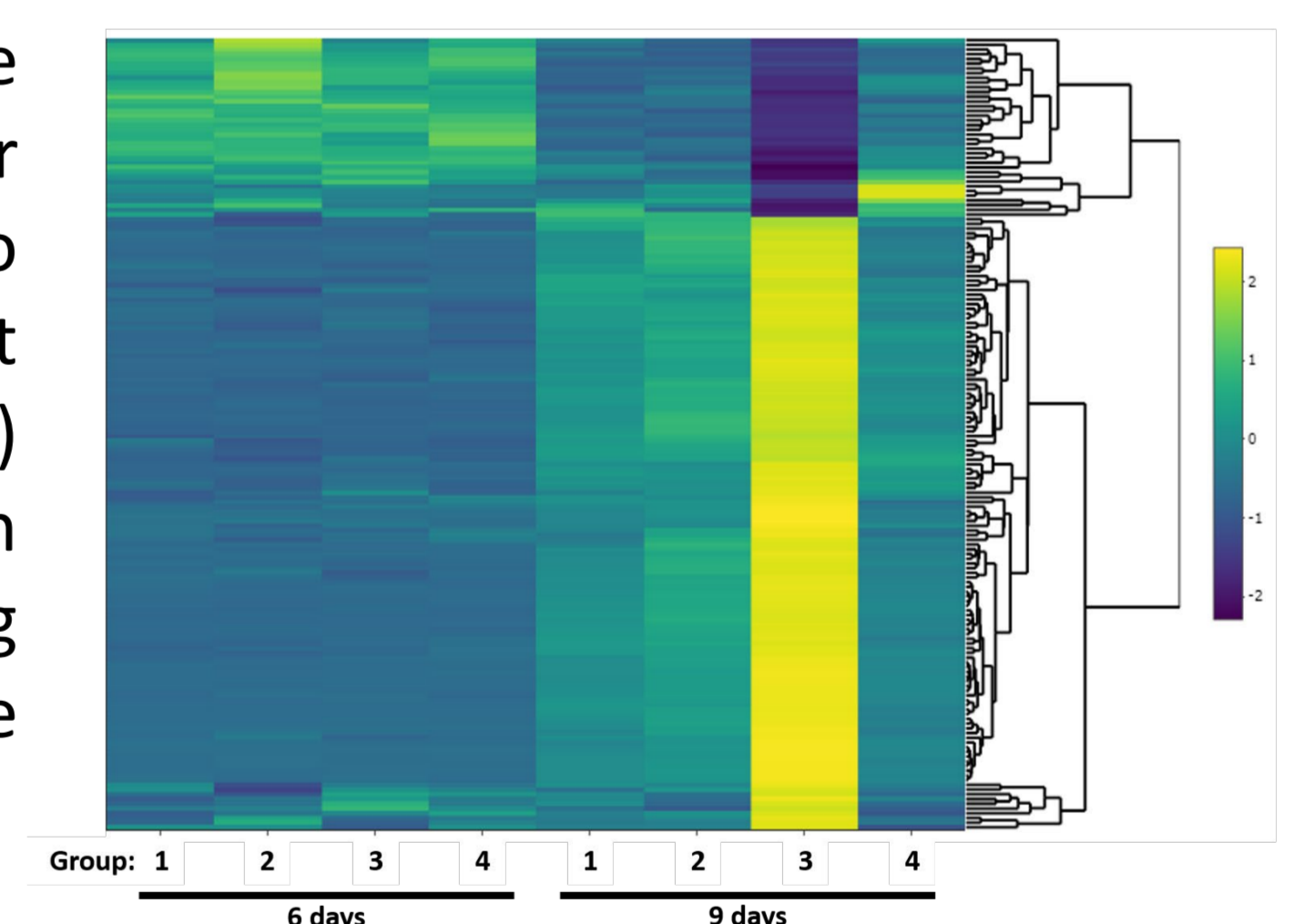
### 2. Oocyst growth affects developmental trajectory

Oocysts are separated by day and size on a PCA plot of their transcriptomes. Little difference is seen between day-6 oocysts despite their different nutrient status and sizes, but greater differences are seen between day-9 oocysts.



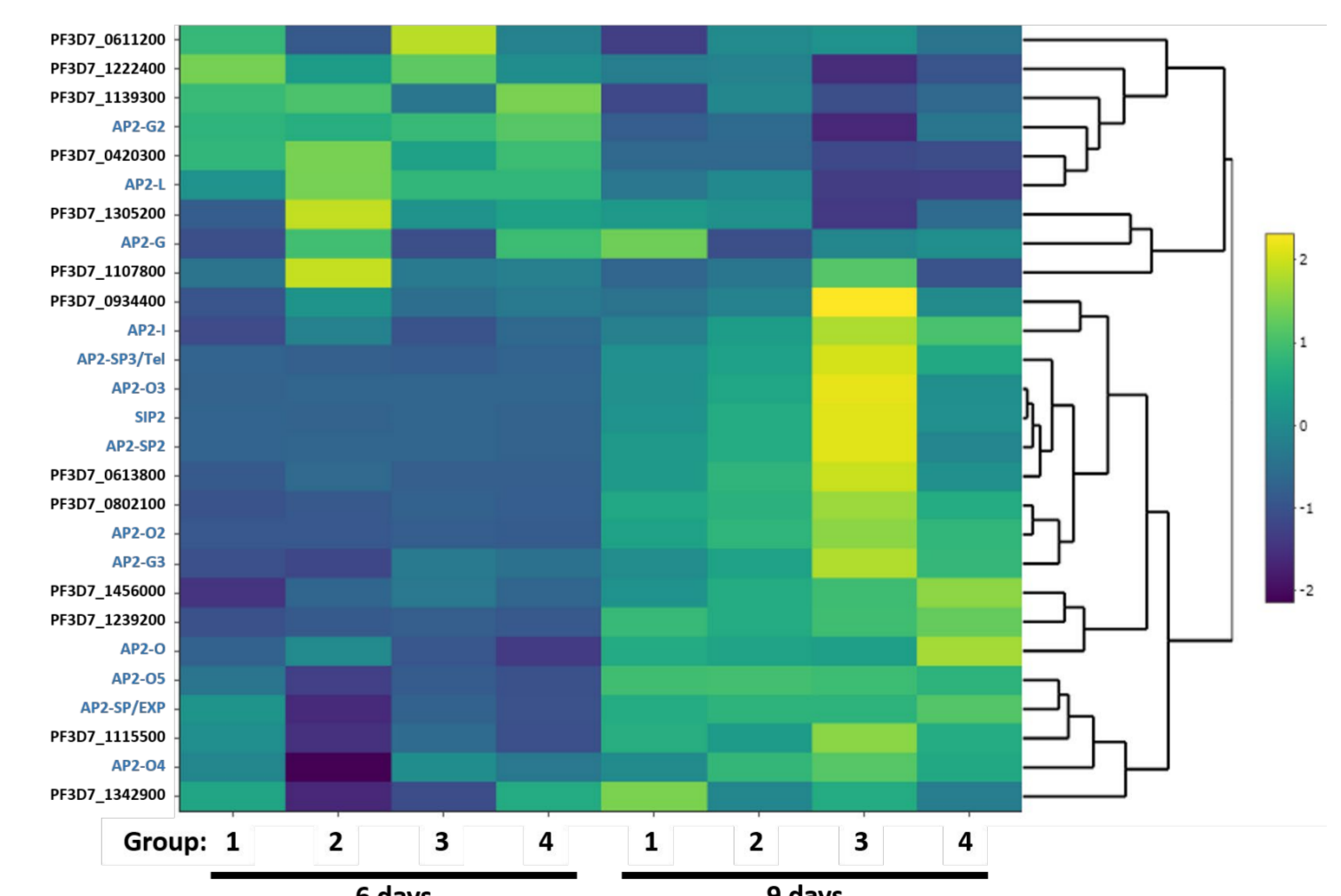
### 3. Two phases of oocyst development

Two phases of oocyst development are seen: 1) growth including nuclear division and 2) differentiation leading to sporozoite production. The most nutrient-boosted oocysts (Group 3) enter sporulation much earlier than oocysts other conditions, expressing proteins involved in sporozoite development and motility.



### 4. A transcriptional program switch

*P. falciparum* encodes 27 AP2 domain-containing transcription factors. Two distinct profiles of AP2 expression are seen at 6 and 9 dpi, with known AP2s directing sporogony upregulated in the most nutrient-boosted oocysts (Group 3), alongside several uncharacterised AP2 transcription factors.



## Conclusions and Outlook

- Oocysts in different nutrient environments exhibit little transcriptional differences 6 dpi despite the clear nutrient impact on growth
  - Nutrient boosting accelerates oocyst switch to differentiation and sporozoite production reflected by major changes in gene expression
  - The two phases of oocyst development are controlled by different transcriptional programmes directed by AP2 transcription factors.
- Moving forward, the great heterogeneity amongst oocysts in any certain condition will be investigated with single cell RNA sequencing.

## References & Acknowledgements

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1. WHO World Malaria Report 2021
2. Vlachou, D et al. (2005). Functional genomic analysis of midgut epithelial responses in *Anopheles* during *Plasmodium* invasion. *Current Biology* - *CB*, 15(13), 1185-1195.
3. Habtewold, et al. (2021). *Plasmodium* oocysts respond with dormancy to crowding and nutritional stress. *Scientific Reports*, 11(1)