Sex-specific genetic screens identify hundreds of \textit{Plasmodium} fertility genes essential for the transmission of malaria parasites

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INTRODUCTION

Malaria parasites reproduce sexually to infect mosquitoes. Blocking transmission has a key role in malaria elimination, but many molecular mechanisms of fertility that could be targeted are poorly understood.

A SEX-SPECIFIC GENETIC SCREEN IN \textit{PLASMODIUM BERGHEI}

Barcoded gene-targeting PlasmoGEM\textsuperscript{2} vectors were used to interrogate >1200 targetable genes\textsuperscript{3} for their roles in fertility. Most fertility genes are sex-specific. To prevent barcode transmission through the opposite sex, single-sex \textit{P. berghei} lines were mutagenised and then crossed with gametocytes of the opposite sex. Mutant barcodes were counted in blood input and oocyst output samples to determine the relative fertility of each mutant.

HUNDREDS OF GENES ESSENTIAL FOR FERTILITY

The relative growth rate of each mutant in the female-only and male-only lines are shown on log2 scales, and genes with known phenotypes are highlighted\textsuperscript{1-12}:

- **Results summary:**
  - 156 genes essential for female fertility
  - 128 genes essential for male fertility
  - 79 genes essential for female and male fertility
  - 685 genes not essential for fertility

SEX-SPECIFIC EXPRESSION OF FERTILITY GENES

Male-specific (11, 12) and female-specific (13, 14) gene clusters defined in the Malaria Cell Atlas\textsuperscript{14} are highlighted in A.

Male fertility genes are significantly enriched in the male-specific clusters, and female fertility genes are significantly enriched in the female-specific clusters and cluster 4, which includes genes expressed in the liver stage, trophozoites, females, ookinetes and oocysts (B).

A MALE MOTILITY SCREEN

Male gametes from a pool of 197 mutants with the strongest male fertility phenotypes were purified (A) and sampled for barcode sequencing to determine mutant type (B). Known and predicted pre-motility\textsuperscript{9,10}, motility\textsuperscript{15-17} and post-motility mutants\textsuperscript{1,5-7,24} are highlighted in the pilot motility screen data (log2 scales; C).

SUMMARY

- 1249 \textit{P. berghei} genes screened of which 405 affect fertility.
- Essential male fertility genes can be predicted by gene expression.
- Pilot male motility screen identifies post-motility mutants.
- Validating unknown proteins will uncover new molecular mechanisms and drug and vaccine targets.