Sex-specific genetic screens identify hundreds of *Plasmodium* fertility genes essential for the transmission of malaria parasites

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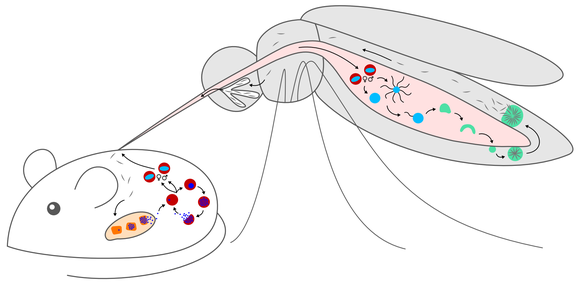
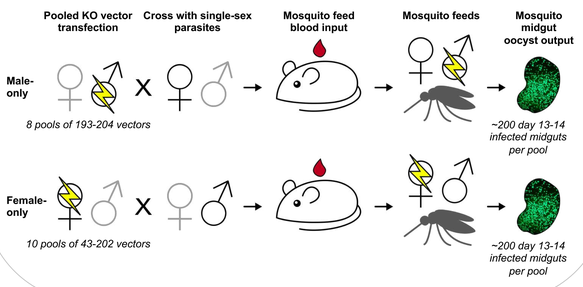
INTRODUCTION

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.



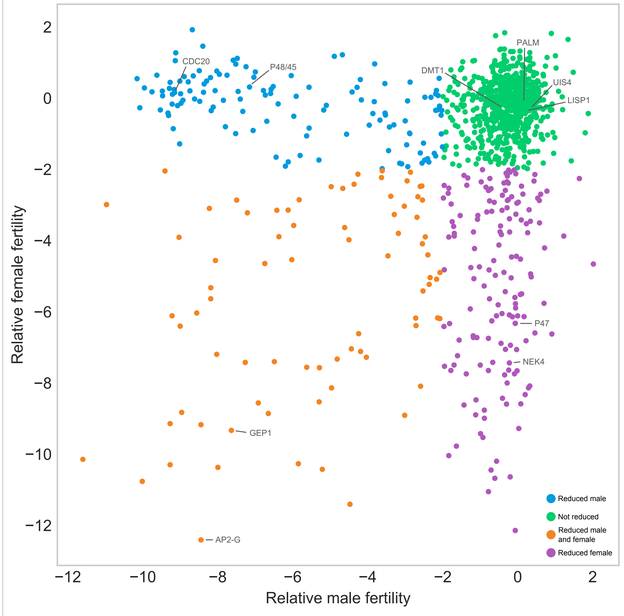
A SEX-SPECIFIC GENETIC SCREEN IN *PLASMODIUM BERGHEI*

Barcoded gene-targeting *Plasmo*GEM[1] vectors were used to interrogate >1200 targetable genes[2] for their roles in fertility. Most fertility genes are sex-specific. To prevent barcode transmission through the opposite sex, single-sex *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[3-13]:



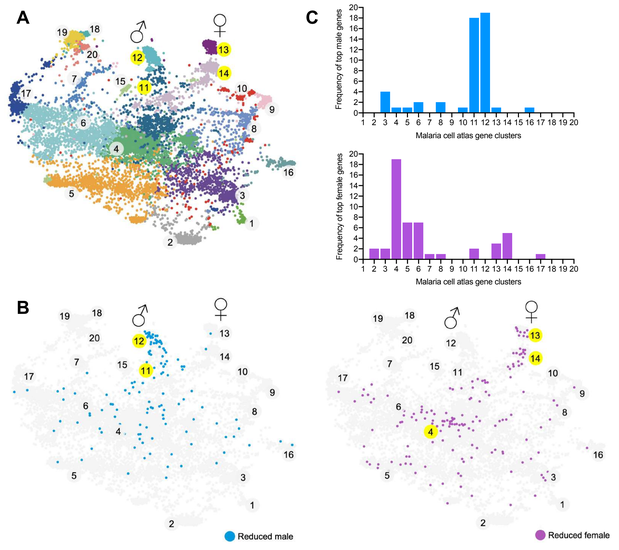
Results summary:

- 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[14] are highlighted in A.

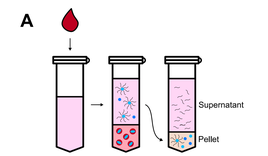
Male fertility genes are significantly enriched in the male-specific clusters, and female fertility genes are significantly enriched in the female-specific clusters and cluster 4, which includes genes expressed in the liver stage, trophozoites, females, ookinetes and oocysts (B).



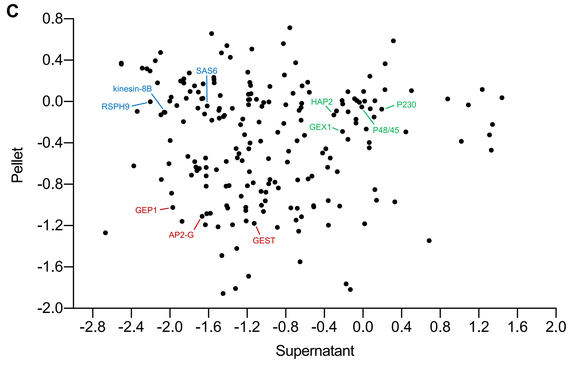
74 % of the top 50 male fertility genes are in the male-specific clusters, and 16 % of the top 50 female fertility genes are in the female-specific clusters (C).

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[8,9,18], motility[15-17] and post-motility mutants[3,5,19,20] are highlighted in the pilot motility screen data (log2 scales; C).



Mutant type	Pel	Sup
Pre-motility	x	x
Motility	✓	x
Post-motility	x	✓



SUMMARY

- 1249 *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.

References: 1. Gomes, A.R. et al., 2015. A genome-scale vector resource enables high-throughput reverse genetic screening in a malaria parasite. *Cell Host Microbe*, 17(3):404-413; **2.** Bushell, E.S. et al., 2017. Functional profiling of a *Plasmodium* genome reveals an abundance of essential genes. *Cell*, 170(2):260-272.e8; **3.** van Dijk, M.R. et al., 2001. A central role for P48/45 in malaria parasite male gamete fertility. *Cell*, 104(1):153-6; **4.** Guttery, D.S. et al., 2012. A putative homologue of CDC20/CDH1 in the malaria parasite is essential for male gamete development. *PLoS Pathog*, 8(2):e1002555; **5.** van Dijk, M.R. et al., 2010. Three members of the 6-cys protein family of *Plasmodium* play a role in gamete fertility. *PLoS Pathog*, 6(4):e1000853; **6.** Reininge, L. et al., 2005. A NIMA-related protein kinase is essential for completion of the sexual cycle of malaria parasites. *J Biol Chem*, 280(36):31957-64; **7.** Khan, S.M. et al., 2005. Proteome analysis of separated male and female gametocytes reveals novel sex-specific *Plasmodium* biology. *Cell*, 121(5):675-87; **8.** Jiang, Y. et al., 2020. An intracellular membrane protein GEP1 regulates xanthurenic acid induced gametogenesis of malaria parasites. *Nat Commun*, 11(1):1764; **9.** Yuda, M. et al., 2015. Global transcriptional repression: An initial and essential step for *Plasmodium* sexual development. *PNAS*, 112(41):12824-9; **10.** Kenthirapalan, S. et al., 2016. Functional profiles of orphan membrane transporters in the life cycle of the malaria parasite. *Nat Commun*, 7:10519; **11.** Haussig, J. et al., 2011. Inactivation of a *Plasmodium* apicoplast protein attenuates formation of liver merozoites. *Mol Microbiol*, 81(6):1511-25; **12.** Mueller, A. et al., 2005. *Plasmodium* liver stage developmental arrest by depletion of a protein at the parasite-host interface. *PNAS*, 102(8):3022-7; **13.** Ishino, T. et al., 2009. LISP1 is important for the egress of *Plasmodium berghei* parasites from liver cells. *Cell Microbiol*, 11(9):1329-39; **14.** Howick, V.M. et al., 2019. The Malaria Cell Atlas: Single parasite transcriptomes across the complete *Plasmodium* life cycle. *Science*, 365(6455):eaaw2619. **15.** Marques, S.R. et al., 2015. An essential role of the basal body protein SAS-6 in *Plasmodium* male gamete development and malaria transmission. *Cell Microbiol*, 17(2):191-206; **16.** Zeeshan, M. et al., 2019. Kinesin-8B controls basal body function and flagellum formation and is key to malaria transmission. *Life Sci Alliance*, 2(4):e201900488; **17.** Sinden, R.E. et al. 2010. The flagellum in malarial parasites. *Curr Opin Microbiol*, 13(4):491-500. **18.** Talman, A.M. et al., 2011. *Pb*GEST mediates malaria transmission to both mosquito and vertebrate host. *Mol Microbiol*, 82(2):462-74; **19.** Liu, Y. et al., 2008. The conserved plant sterility gene HAP2 functions after attachment of fusogenic membranes in *Chlamydomonas* and *Plasmodium* gametes. *Genes Dev*, 22(8):1051-68; **20.** Ning, J. et al., 2013. Comparative genomics in *Chlamydomonas* and *Plasmodium* identifies an ancient nuclear envelope protein family essential for sexual reproduction in protists, fungi, plants, and vertebrates. *Genes Dev*, 27(10):1198-215.

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