Dimorphism in genes encoding sexual-stage proteins of *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*\(^*\)

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**A R T I C L E  I N F O**

**Abstract**

*Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* are distinct species of malaria parasite which are sympatric throughout the tropics, except for the Americas. Despite this complete overlap in geographic range, these two species do not recombine. Although morphologically very similar, the two taxa must possess distinct characters which prevent recombination between them. We hypothesised that proteins required for sexual reproduction have sufficiently diverged between the two species to prevent recombination in any mosquito blood meal in which gametocytes of both species are ingested. In order to investigate possible barriers to inter-species mating between *P. ovale curtisi* and *P. ovale wallikeri*, homologues of genes encoding sexual stage proteins in other plasmodia were identified and compared between the two species. Database searches with motifs for 6-cysteine, *Limulus* Coagulation factor C domain-containing proteins and other relevant sexual stage proteins in the genus *Plasmodium* were performed in the available *P. ovale curtisi* partial genome database (Wellcome Trust Sanger Institute, UK). Sequence fragments obtained were used as the basis for PCR walking along each gene of interest in reference isolates of both *P. ovale curtisi* and *P. ovale wallikeri*. Sequence alignment of the homologues of each gene in each species showed complete dimorphism across all isolates. In conclusion, substantial divergence between sexual stage proteins in the two *P. ovale* spp. was observed, providing further evidence that these do not recombine in nature. Incompatibility of proteins involved in sexual development and fertilisation thus remains a plausible explanation for the observed lack of natural recombination between *P. ovale curtisi* and *P. ovale wallikeri*.

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**1. Introduction**

Ovale malaria in humans is caused by *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*, which were recently differentiated (Sutherland et al., 2010) and found to be sympatric in their distribution across several communities in Africa and Asia (Oguike et al., 2011; Bauffe et al., 2012; Fuehrer et al., 2012; Putaporntip et al., 2013). Although occurring together, these two species do not recombine in nature and are genetically distinct at all loci examined to date. Phylogenetic analysis based on only a few loci suggests that divergence between *P. ovale curtisi* and *P. ovale wallikeri* is likely to have occurred 1–5 million years ago. This genetic distance may reflect evolutionary isolation (Sutherland and Polley, 2011) as the most likely explanation for lack of recombination. *Plasmodium ovale curtisi* and *P. ovale wallikeri* are sometimes found co-infecting a single individual (Fançon et al., 2012; Fuehrer et al., 2012), thus providing compelling evidence for true biological separation and consequently their inability to mate. *Plasmodium ovale* spp. often circulate in the population at submicroscopic levels, frequently as mixed infections with *Plasmodium falciparum*, *Plasmodium vivax* or *Plasmodium malariae*, and so pose a challenge to malaria control and elimination (Fançon et al., 2012; Dinko et al., 2013). These usually asymptomatic infections make a contribution to the overall malaria transmission burden, and thus a better understanding of the sexual stage biology of ovale malaria is needed to explain the observed pattern of transmission of these parasites.

Fertilisation and development of the malaria parasite in the mosquito midgut is a crucial process for malaria transmission,
which in *P. falciparum* can be interrupted by human antibodies against a number of sexual stage proteins (Ouedraogo et al., 2011), and several of these have been investigated as potential malaria transmission-blocking vaccine antigens. PfS230 and PfS48/45 belong to the 6-cysteine family and are proteins expressed on the surface of gametes (Carter et al., 1995; van Dijk et al., 2001, 2010). CCP/LAP proteins are another group of antigens expressed during gametocyte differentiation and gametogenesis (Delrieu et al., 2002; Pradel et al., 2004; Scholz et al., 2008) which, when disrupted, lead to blockage of the translation of sporozoites from the oocyst on the mosquito midgut wall to the salivary gland. These proteins are highly conserved in apicomplexan parasites and consist of multiple predicted adhesive domains, including a common *Limulus* Coagulation factor C (LCCL) domain (Dessens et al., 2004; Pradel et al., 2004; Templeton et al., 2004). Pfg377 is a gametocyte-specific protein expressed during the development of osmophilic bodies, which are thought to be essential for the emergence of female gametocytes from red blood cells to allow fertilisation with the short-lived male gametes in the mosquito midgut (Severini et al., 1999; de Koning-Ward et al., 2008). The gene is located on chromosome 12 in *P. falciparum* and is expressed as protein in stage III gametocytes. *Plasmodium* meiosis occurs in the mosquito after fertilisation between male and female gametes, and requires a variety of well-conserved components. Rad51 and Dmc1 (disrupted meiotic cDNA), eukaryotic homologues of the *Escherichia coli* RecA protein, play a critical role in homologous DNA strand exchange reaction during meiotic recombination and repair (Copalakrishnan and Kumar, 2013) in malaria parasites.

The publication of complete genome sequences for certain *Plasmodium* spp. (Gardner et al., 2002; Hall et al., 2005) has provided an opportunity for better understanding of parasite biology. Thus far, only partial genome sequence data based on a single Nigerian isolate of *P. ovale curtisi*, collected in the 1970s and propagated in captive chimpanzees, is publicly available. This incomplete dataset can be accessed for BLAST searches on the Wellcome Trust Sanger Institute server (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/p.ovale) to find *P. ovale*-specific homologues. Primers were designed to target specific *P. ovale* sequences obtained (Table 1). For a putative LCCL domain-containing protein, after obtaining approximately the first 2 kb fragment, a further search was done with the sequence in the ovale database resulting in an extended ~5 kb contig assembled from various partial sequences. Since the available ovale sequence database is from *P. ovale curtisi*, corresponding *P. ovale wallikeri* sequences were obtained after amplification of genomic DNA from isolates of both species and direct sequencing. Comparison between *P. ovale curtisi* and *P. ovale wallikeri* sequences generated from all putative fertilisation genes was performed using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

### 2. Materials and methods

#### 2.1. Patients

Initial characterisation of loci of interest was performed on two parasite isolates previously obtained with written informed consent from patients treated for ovale malaria at University College London Hospital, UK in 2010 (UK Health Research Authority National Research Ethics reference 07/Q0505/60). *Plasmodium ovale curtisi* isolate HL1002Poc was obtained from a Ugandan-born woman of 51 years, permanently resident in the UK, who reported several overseas journeys in the 3 years from 2007 to 2010. The patient presented with symptoms of malaria at the beginning of October, 2010, after having spent 1 week in Sierra Leone in September 2010. No conventional chemoprophylaxis was used, but a “herbal formula” (not further specified) was taken for malaria prevention while travelling. *Plasmodium ovale wallikeri* isolate HL1001Pw was obtained from a British male, aged 18 years, who returned to the UK in August 2010 from 1 month charity work in coastal Ghana. He reported good compliance with atovaquone-proguanil prophylaxis including the complete 7-day post-exposure period and use of mosquito protection, but experienced the first malaria symptoms 5 weeks after arriving back in the UK. This suggests the isolate came from a liver-stage relapse without a prior primary blood-stage infection due to effective chemoprophylaxis, as previously described (Nolder et al., 2013).

Genomic DNA preparations from archived *P. ovale* spp. blood samples from the Public Health England Malaria Reference Laboratory (PHE MRL), UK, previously confirmed as either *P. ovale curtisi* (*n* = 39) or *P. ovale wallikeri* (*n* = 39) by Nolder et al. (2013), were also available for this study (Table 1).

#### 2.2. Informatic and laboratory procedures

BLASTX searches using amino acid sequences from LCCL domain-containing proteins, PfS48/45, PfS230, Pfg377 and DMC1-like protein, were performed in the *P. ovale curtisi* fragment database (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/p.ovale) to find *P. ovale*-specific homologues. Primers were designed to target specific *P. ovale* sequences obtained (Table 2). For a putative LCCL domain-containing protein, after obtaining approximately the first 2 kb fragment, a further search was done with the sequence in the ovale database resulting in an extended ~5 kb contig assembled from various partial sequences. Since the available ovale sequence database is from *P. ovale curtisi*, corresponding *P. ovale wallikeri* sequences were obtained after amplification of genomic DNA from isolates of both species and direct sequencing. Comparison between *P. ovale curtisi* and *P. ovale wallikeri* sequences generated from all putative fertilisation genes was performed using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

### 3. Results

We identified the *P. ovale curtisi* and *P. ovale wallikeri* homologues of genes encoding known sexual-stage proteins in other members of the genus *Plasmodium* and determined their DNA sequence in order to quantify any divergence between the two ovale species. Using BLASTX searches, homologues of five genes encoding known sexual-stage proteins in the genus *Plasmodium* were identified in *P. ovale curtisi*. Corresponding orthologues of each gene were successfully amplified from *P. ovale wallikeri* genomic DNA and sequenced as previously described (Sutherland et al., 2010). Data obtained are summarised in Table 3. All novel sequences have been deposited in GenBank with accession numbers KP690796, KP690797, KP725066, KP725067, KP749432–KP749437.
Table 2
Primers and cycling conditions. A list of all five genes with primer sequences used for PCR and sequencing are shown, including PCR product sizes and cycling conditions.

| Gene     | Primers 1 | Primer sequence | PCR product size | PCR conditions |
|----------|-----------|-----------------|------------------|---------------|
| Pos230   | Po-398 F1 | 5'-AAT GGA GGA AAG ACA AAG GGA G-3' | 643 bp | 95°C/15°C/52°C/20°C/68°C/34°C/68°C |
|          | Po-398 R1 | 5'-GTT GGT CAT AGT AGT ATG CTC-3' | 290 bp | 95°C/15°C/48°C/20°C/68°C/30°C/68°C |
|          | Po-398 F3 | 5'-TCA AGA GAT AAA TAT GTC-3' | 598 bp | 95°C/15°C/52°C/24°C/72°C/1°C/72°C |
|          | Po-398 R3 | 5'-GAA ATC TCA TTA AGA GTA CTC-3' | 343 bp | 95°C/15°C/52°C/24°C/72°C/29°C/72°C |
| Po12     | Po-161 F1 | 5'-GAC CTT CCC TAC TAC CCA GAA-3' | 2408 bp | 94°C/15°C/55°C/68°C/38°C/30°C/39°C/68°C |
|          | Po-161 R1 | 5'-GTT TGT GAA GAT GGG GAT G-3' | 1934 bp | 94°C/15°C/55°C/68°C/38°C/30°C/39°C/68°C |
|          | Po-161 R3 | 5'-AGC AAG TGC AGA TGG AAG AGG-3' | 944 bp | 95°C/15°C/54°C/68°C/38°C/30°C/39°C/68°C |

LCCL Domain

| Primers 1 | Primer sequence | PCR product size | PCR conditions |
|-----------|-----------------|------------------|---------------|
| LCF1      | 5'-GCC CCT CTA CTT GCT GTA AGA C-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
|           | 5'-ATG AAC CCT GCT CAT GTA TTA G-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |

(LCP1)

| Primers 1 | Primer sequence | PCR product size | PCR conditions |
|-----------|-----------------|------------------|---------------|
| LCP1      | 5'-CAAT CCA CAT CCA GTA TCA C-3' | 944 bp | 95°C/15°C/54°C/68°C/38°C/30°C/39°C/68°C |

PoRad51

| Primers 1 | Primer sequence | PCR product size | PCR conditions |
|-----------|-----------------|------------------|---------------|
| PoRad Fl  | 5'-GTT GTA ACA AGT AAA CCA G-3' | 710 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
|           | 5'-GAA ATC TCA CTT CAT CAT CCA-3' | 663 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
| PoRad R1  | 5'-AGG AAG ATC TAC TCC CCA AGC-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
| PoRad R2  | 5'-ATG CAA TCC ATT GAT AGT TAG-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
| PoRad F1  | 5'-TTG GTA ACA TTA AAT AGA TTA CAT C-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
|           | 5'-GCC CCT CTT CTA CTT GTA AGA C-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
|           | 5'-ATG AAC CCT GCT CAT GTA TTA G-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |

Pow419 (Pog377)

| Primers 1 | Primer sequence | PCR product size | PCR conditions |
|-----------|-----------------|------------------|---------------|
| Po-419 Fl | 5'-CAG AAT CAA CTA GTA TAA AAG AGA-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
| Po-419 R1 | 5'-TCA ATC TCA TGA AAG AGA CTA A-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
| Po-419 F2 | 5'-GTT GGT TTA GAA TGA TGG AAG AG-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |
| Po-419 R2 | 5'-GCC CCT CTA CTT GCT GTA AGA C-3' | 670 bp | 95°C/15°C/50°C/65°C/35°C/65°C |

LCCL F1 & F3 sequencing primers

| Primers 1 | Primer sequence | PCR product size | PCR conditions |
|-----------|-----------------|------------------|---------------|
| LCP1      | 5'-GCC CCT CTT CTA CTT GTA AGA C-3' | 944 bp | 95°C/15°C/54°C/68°C/38°C/30°C/39°C/68°C |
|           | 5'-ATG AAC CCT GCT CAT GTA TTA G-3' | 944 bp | 95°C/15°C/54°C/68°C/38°C/30°C/39°C/68°C |

LCCL 11 & 3 primers

| Primers 1 | Primer sequence | PCR product size | PCR conditions |
|-----------|-----------------|------------------|---------------|
| LCP1      | 5'-GCC CCT CTT CTA CTT GTA AGA C-3' | 944 bp | 95°C/15°C/54°C/68°C/38°C/30°C/39°C/68°C |
|           | 5'-ATG AAC CCT GCT CAT GTA TTA G-3' | 944 bp | 95°C/15°C/54°C/68°C/38°C/30°C/39°C/68°C |

Table 3
Five sexual stage genes were sequenced in Plasmodium ovale curtisi (Poc) and Plasmodium ovale wallikeri (Pow). The number of isolates sequenced for each gene and their codons relative to that of Plasmodium falciparum 3D7 are shown. For the purposes of this study only the “Reference” sequence for these sexual stage loci was that obtained for each gene from the first isolate sequenced. This was isolate Z0015 for P. ovale curtisi and Z0014 for P. ovale wallikeri.

| Genes sequenced | PocCp1 | Pog377 | Pos230 | Po12 | PoRad51 |
|-----------------|--------|--------|--------|------|---------|
| Sequence length analysed | 1586 aa | 192 aa | 97 aa | 114 aa | 233 aa |
| P. falciparum homologue (codons) | | | | | |
| FF037_D1745500 (1-1620) | FF037_D1250100 (367-557) | FF037_D0209000 (885-986) | FF037_D0612700 (120-236) | FF037_D1107400 (1-233) |
| Full-length | Yes | No | No | No | No |
| Observed non-synonymous differences Poc vs Pow | 35 aa | 18 aa | 4 aa | 4 aa | 0 aa |
| No. identical to reference (No. sequenced) | 4 (4) | 6 (6) | 40 (40) | 40 (40) | 7 (7) |

aa, amino acids; nt, nucleotides.

Sequence alignment of P. ovale curtisi and P. ovale wallikeri for each gene demonstrated perfect dimorphism between the two species, with up to 40 independent isolates of each species analysed (Table 3). Best scoring homologues by sequence identity for PocCp1, Pos230, Pog377 and PoRad51 were Plasmodium knowlesi LCC domain-containing protein, Plasmodium chabaudi 6-cysteine protein (230), Plasmodium yoelii osmophilic body protein (G377) and P. falciparum Rad51, respectively.

3.1. PocCp1 and PogCp1

Full-length sequences for pocCp1 and powCp1, encoding amino acids (aa) 1–1586 of the LCCL-domain protein PCCP1, were isolated by a combination of draft sequence contig assembly and PCR “walking” along the gene for both parasite species. A total of eight samples were fully sequenced. PocCp1 and PowCp1 are similar to each other and show good sequence identity to
orthologues in six other species in the genus (Supplementary Fig. S1). Despite their sequence similarity, the genes from the two ovale species differed from each other at 35 of 1586 aa positions (2.2%) and the four isolates of each species from which we derived full-length sequence were identical. For comparison, the two rodent malaria orthologues most similar to each other, P. yoelli and Plasmodium berghei, differed by 55 aa over a total of 1615 (3.4%) (Supplementary Fig. S1).

3.2. Poc_g377 and Pow_g377

A contig encoding 193 aa of both ovale species was obtained, corresponding to codons 367–557 of the P. falciparum (3D7 reference genome) ortholog PF3D7_1250100, which has a coding region of 3119 aa. A total of 12 samples were evaluated. Nineteen of the 193 amino acids sequenced (9.8%) differed between P. ovale curtisi and P. ovale wallikeri, suggesting substantial inter-species heterogeneity (Fig. 1). Plasmodium yoelli and P. berghei also differ from each other by 19 aa in this region of the gene. To understand how this compares to intra-species diversity, we examined publicly available whole genome data for over 600 P. falciparum isolates across this 200 aa segment, using the PlasmoView freeware program (Preston et al., 2014). We could identify only eight non-synonymous nucleotide substitutions between any two isolates of P. falciparum.

3.3. Poc_s230 and Pow_s230

Pfs230, a member of the 6-cysteine gene family, plays a crucial role in the sexual stages of Plasmodium development (van Dijk et al., 2010) and shows significant inter-species divergence apart from the conserved cysteine-rich domains (Fig. 2A). A total of 80 samples were evaluated. The orthologues in P. ovale curtisi and P. ovale wallikeri were more similar in sequence to each other than to other members of the genus, but four non-synonymous differences between the ovale species were observed in the 97 codon segment analysed here.

3.4. Poc_p12 and Pow_p12

P12 is a member of the 6-cysteine gene family but not implicated in the sexual stages of Plasmodium development (Li et al., 2012). It also shows significant inter-species divergence between the P fg377 and Rad51 in sexual development, fertilisation and ookinite formation (Fig. 2B). A total of 80 samples were evaluated. As for Po_s230, the P12 orthologues between the conserved cysteine-rich domains (Fig. 1). Plasmodium yoelli and P. berghei also differ from each other by 19 aa in this region of the gene. To understand how this compares to intra-species diversity, we examined publicly available whole genome data for over 600 P. falciparum isolates across this 200 aa segment, using the PlasmoView freeware program (Preston et al., 2014). We could identify only eight non-synonymous nucleotide substitutions between any two isolates of P. falciparum.

3.5. Poc_rad51 and Pow_rad51

A total number of 13 samples were evaluated. The Rad51 protein, which contributes to maintenance of chromosome strand sequence fidelity during replication, is highly conserved across the genus, and there were no differences between the 233 aa predicted from the DNA segment sequenced in P. ovale curtisi and those predicted by the corresponding segment of the gene in P. ovale wallikeri (Table 3). However, differences were present at the nucleotide level, with seven synonymous nucleotide differences between the two species present in this partial gene sequence.

4. Discussion

In this study, we identified significant dimorphism in genes of P. ovale curtisi and P. ovale wallikeri encoding protein orthologues to sexual stage proteins in other Plasmodium spp. All P. ovale curtisi sequences were identical to each other across the five gene segments studied; the same was true for P. ovale wallikeri. Previous studies (Pradel et al., 2004; de Koning-Ward et al., 2008; van Dijk et al., 2010; Gopalakrishnan and Kumar, 2013) have shown the role of PfS230, Pf48/45, LCCL domain-containing proteins, Pfg377 and Rad51 in sexual development, fertilisation and ookinite formation. Although these have been identified and studied in other Plasmodium spp., we believe this is the first study of these particular genes in P. ovale spp. An ~5 kb region of the gene encoding the Ccp1 protein was sequenced in P. ovale curtisi and P. ovale wallikeri and 36 single nucleotide polymorphisms (SNPs) were observed between them. Although the other loci investigated were smaller in size, substantial dimorphisms were also obtained between the P. ovale spp, particularly in the gene for Pos377, which exhibited only 90% sequence identity at the amino acid level between the two ovale species. This striking dimorphism between P. ovale curtisi and P. ovale wallikeri is consistent with previous studies of a variety of loci (reviewed by Fuehrer and Noedl, 2014). Although some intra-species heterogeneity has been
observed in genes encoding Cox1, TRA, MSP1 and the sssRNA, from one or both species, in each case the alleles sequenced belonged clearly to the appropriate dimorph (Sutherland et al., 2010; Fuehrer et al., 2012; Putaporntip et al., 2013; Tanomsing et al., 2013). One or both species, in each case the alleles sequenced belonged clearly to the appropriate dimorph (Sutherland et al., 2010; Fuehrer et al., 2012; Putaporntip et al., 2013; Tanomsing et al., 2013).

Recent evidence (Nolder et al., 2013) has indicated a significant difference in relapse patterns between P. ovale curtisi and P. ovale wallikeri in ovale malaria cases imported to the UK. This is the first known description of a clinically important feature which differs between these two genetically distinct species, but does not necessarily explain the separation between them. It is accepted that for true speciation to occur, two populations from an ancestral species must become reproductively isolated, thereby preventing gene flow between them (Sutherland and Polley, 2011). Presumably, this is the case for P. ovale curtisi and P. ovale wallikeri, which are sympatric across their range and thus are not separated by geographical barriers. Biological barriers to genetic recombination could arise by various mechanisms; for example temporal segregation could occur through a shift in the timing of gamete release, and genetic segregation could occur through accrual of mutations in gamete surface receptors that might prevent viable fertilisation. It is likely that the separation of the two ovale lineages was further exacerbated by the timing of ancestral host transitions into hominids, allowing a lengthy period in different primate hosts during which incompatibility in fertilisation biology could have developed (Sutherland and Polley, 2011).

The most striking divergence between P. ovale curtisi and P. ovale wallikeri in our analysis was in the gene variants encoding Po_g377, a protein expressed only in female gametocytes (in other Plasmodium spp.) and localised to the osmiophilic bodies (de Koning-Ward et al., 2008). The divergence of these putative sexual stage molecules between the two P. ovale spp. may have contributed to their inability to mate. Mutations within incompatible loci are known to produce genetic isolations within sympatric populations of animals and plants (Palumbi and Metz, 1991; Amato et al., 2007). As discussed above, ovale parasites exhibit minimal intra-species heterogeneity and it remains possible that P. ovale curtisi and P. ovale wallikeri may essentially propagate clonally with frequent self-fertilization (Sutherland and Polley, 2011; Putaporntip et al., 2013).

Not all of the protein coding genes investigated showed great diversity between the two ovale species. Ps230 is the largest representative, and P12 the smallest, of a 10-member family of proteins found in all Plasmodium spp. The family is defined by partially conserved, cysteine-rich "double domains" that are approximately 350 aa in length and have one to three predicted disulfide bridges in each half. These domains are important features for protein folding in this family and are characterised by motifs of two cysteine molecules spanned by two other amino acids (Dessens et al., 2004). This results in a specific tertiary structure which is conserved among species. Ps230, with seven double domains, is expressed on the gamete surface and is described as a transmission-blocking vaccine candidate antigen (Gerloff et al., 2005). Comparison based on sequence identities in the putative Pos230 and other gene loci investigated suggest a closer relationship between P. ovale spp. and the rodent species P. yoelli, P. berghei, P. chabaudi, but less similarity to P. falciparum. Neither of these genes were as divergent between the ovale species as the Pg_s377 genes. Similarly, for Rad51, a highly conserved chromosome repair protein associated with DNA replication at mitosis and meiosis, only a handful of synonymous sequence differences were observed between P. ovale curtisi and P. ovale wallikeri. Recent advances in molecular diagnostics and species discrimination have provided further insight on the global distribution of these two species and all evidence to date is wholly consistent with non-recombination between them (Fuehrer et al., 2012; Putaporntip et al., 2013).

In conclusion, we have observed that substantial dimorphism exists between putative sexual stage proteins in the two P. ovale spp. adding yet further evidence that these two species do not recombine in nature. Gametocytes are often observed in peripheral blood smears in ovale malaria patients but it is unclear how this relates to P. ovale curtisi and P. ovale wallikeri transmissibility. The data herein provide a platform for gene expression studies to further investigate the transmission biology of ovale malaria.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2015.02.004.

References

Amato, A., Koostora, W.H.C.F., Ghiron, J.H.L., Mann, D.G., Proschold, T., Montresor, M., 2007. Reproductive isolation among sympatric cryptic species in marine diatoms. Protist 158, 193–207.
Bauffe, F., Desplams, J., Fraissier, C., Parzy, D., 2012. Real-time PCR assay for discrimination of Plasmodium ovale curtisi and Plasmodium ovale wallokieri in the Ivory Coast and in the Comoros Islands. Malar. J. 11, 307.
Carter, R., Coulton, A., Bhatti, S., Taylor, B.J., Elliott, J.F., 1995. Predicted disulphide-bonded structures for three uniquely related proteins of Plasmodium falciparum. PFG230, PFG48/45 and PFG12. Mol. Biochem. Parasitol. 71, 203–210.
Deoning-Ward, T.F., Olivieri, A., Bertuccini, L., Hood, A., Silvestrini, F., Charvalias, K., Berzosa Diaz, P., Camarda, M., McElwain, T.F., Papenfuss, T., Healer, J., Baldassarri, L., Crabb, B.S., Alano, P., Ranford-Cartwright, L.C., 2008. The role of osmiophilic bodies and Pfg377 expression in female gametocyte emergence and mosquito infectivity in the human malaria parasite Plasmodium falciparum. Mol. Microbiol. 67, 278–290.
Delrieu, I., Waller, C.C., Mota, M.M., Grainger, M., Langhorne, J., Holder, A.A., 2002. Genome sequence of the human malaria parasite Plasmodium ovale curtisi. Mol. Biochem. Parasitol. 121, 11–20.
Dessens, J.T., Sinden, R.E., Claudianos, C., 2004. LCCL proteins of apicomplexan parasites. Trends Parasitol. 20, 102–108.
Dinko, B., Oguike, M.C., Larbi, J.A., Bousema, T., Sutherland, C.J., 2013. Persistent Plasmodium ovale curtisi and Plasmodium ovale wallokieri circulate simultaneously in African communities. Int. J. Parasitol. 43, 677–683.
Djimde, A.A., Doumbo, O.K., Nosten, F., Fairhurst, R.M., Conway, D.J., Roper, C., Bousema, T., Sauerwein, R.W., Waters, A.P., Janse, C.J., 2013. An observational study of malaria in British travellers: Plasmodium ovale wallokieri and Plasmodium ovale curtisi differ significantly in the duration of latency. BMJ Open 3 (3), e002711. http://dx.doi.org/10.1136/bmjopen-2013-002711.
Oguike, M.C., Betsou, M., Burke, M., Nolder, D., Stothard, J.R., Kleinjanschmidt, I., Proietti, C., Bousema, T., Ndongoua, M., Tanabe, K., Ntege, E., Culleton, R., Sutherland, C.J., 2011. Plasmodium ovale curtisi and Plasmodium ovale wallokieri circulate simultaneously in African communities. Int. J. Parasitol. 41, 677–683.
Gerloff, D.L., Creasey, A., Maslau, S., Carter, R., 2005. Structural models for the Plasmodium falciparum replication protein A in Rad51-Rad54-Mediated DNA recombination in Plasmodium falciparum. MBio 4 (3). http://dx.doi.org/10.1128/mBio.00252-13.
Hall, N., Karras, M., Raine, J.D., Carlton, J.M., Kooi, T.W., Berriman, M., Flornes, L., Janssen, C.S., Pain, A., Christophides, G.K., James, K., Rutherford, K., Harris, B., Harris, D., Churcher, C., Quail, M.A., Ormond, D., Doggett, J., Trueman, H.E., Mendoza, J., Bidwell, S.L., Rajandram, M.A., Carucci, D.J., Yates 3rd, J.R., Kafatos, F.C., Janse, C.J., Barrell, B., Turner, C.M., Waters, A.P., Sinden, R.E., 2005. A comprehensive survey of the Plasmodium life cycle by genomic, transcriptomic, and proteomic analyses. Science 307, 82–86.
Li, J., Ito, D., Chen, J.H., Li, F., Cheng, Y., Wang, B., Ha, K.S., Cao, J., Torii, M., Sattabongkot, J., Tsuboi, T., Han, E.T., 2012. Pfv12, a 6-Cys antigen of Plasmodium vivax, is localized to the merozoite rhoptry. Parasitol. Int. 61, 443–449.
Nolder, D., Oguike, M.C., Maxwell-Scott, H., Niayzi, H.A., Smith, V., Chiodini, P.L., Sutherland, C.J., 2013. An observational study of malaria in British travellers: Plasmodium ovale wallokieri and Plasmodium ovale curtisi differ significantly in the duration of latency. BMJ Open 3 (3), e002711. http://dx.doi.org/10.1136/bmjopen-2013-002711.
Ouiattara, J.C., Soudan, A.Z., Traore, B., Ouedraogo, N., Soudan, A.S., 2002. Plasmodium falciparum and Plasmodium ovale osmiophilic bodies and Pfg377 expression in female gametocyte emergence and mosquito infectivity in the human malaria parasite Plasmodium falciparum. Mol. Microbiol. 67, 278–290.
Peterson, J.D., Angiuoli, S., Pertea, M., Allen, J., Selengut, J., Haft, D., Mather, M.W., Fields, K., Salzberg, S.L., Craig, A., Kyes, S., Shallom, S.J., Suh, B., Pain, A., Nelson, K.E., Bowman, S., Paulsen, I.T., James, K., Eisen, J.A., Rutherford, K., Palumbi, S.R., Metz, E.C., 1991. Strong reproductive isolation between closely related sea urchins (genus Echinometra). Mol. Biol. Evol. 8, 227–239.
Pradel, R., Barreto, K., Aravind, M.S., Carucci, D., Schierwater, B., Rinehart, K., Berzosa Diaz, P., Camarda, M., McElwain, T.F., Papenfuss, T., Healer, J., Baldassarri, L., Crabb, B.S., Alano, P., Ranford-Cartwright, L.C., 2008. The role of osmiophilic bodies and Pfg377 expression in female gametocyte emergence and mosquito infectivity in the human malaria parasite Plasmodium falciparum. Mol. Microbiol. 67, 278–290.