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Characterizing the genetic diversity of the monkey malaria parasite *Plasmodium cynomolgi*

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ABSTRACT

Plasmodium cynomolgi is a malaria parasite that typically infects Asian macaque monkeys, and humans on rare occasions. *P. cynomolgi* serves as a model system for the human malaria parasite *Plasmodium vivax*, with which it shares such important biological characteristics as formation of a dormant liver stage and a preference to invade reticulocytes. While genomes of three *P. cynomolgi* strains have been sequenced, genetic diversity of *P. cynomolgi* has not been widely investigated. To address this we developed the first panel of *P. cynomolgi* microsatellite markers to genotype eleven *P. cynomolgi* laboratory strains and 18 field isolates from Sarawak, Malaysian Borneo. We found diverse genotypes among most of the laboratory strains, though two nominally different strains were found to be genetically identical. We also investigated sequence polymorphism in two erythrocyte invasion gene families, the reticulocyte binding protein and Duffy binding protein genes, in these strains. We also observed copy number variation in *rbp* genes.

1. Introduction

The apicomplexan parasite *Plasmodium cynomolgi* causes malaria in Asian monkeys as well as experimental and rare zoonotic infections in humans (Coatney et al., 1971; Eyles et al., 1960; Garnham, 1966; Ta et al., 2014). It shares important biologic features with its sister taxon, the human malaria parasite *Plasmodium vivax*, including a dormant liver stage (Krotoski et al., 1982a; Krotoski et al., 1982b), a preference for invading immature red blood cells (Warren et al., 1966), early formation of infectious sexual stages (Dissanaike et al., 1965), modifications of the infected erythrocyte membrane known as Schuffner's stippling (Aikawa et al., 1975), and tertian periodicity (Mulligan, 1935). *P. vivax* is a serious global health problem that threatens more than 50% of the world's population (Guerra et al., 2010). While *P. vivax* cannot be cultured *in vitro*, severely hampering laboratory studies, *P. cynomolgi* has been successfully adapted to short-term *in vitro* culture (Nguyen-Dinh et al., 1981). Thus *P. cynomolgi* represents an ideal model system for investigating *P. vivax* biology, evolution, and pathology, and for identifying novel drugs against the dormant liver stage.

P. cynomolgi was first described by Mayer in 1907, though this 'type strain' was not preserved (Mayer, 1907). In 1935 H.W. Mulligan comprehensively re-described the species and maintained his isolate as the first laboratory strain (Mulligan, 1935). A second laboratory strain of *P. cynomolgi* was described and established by Garnham in 1959, to which he gave subspecies status as *P. cynomolgi bastianellii* (Garnham, 1959). In the decades following its isolation, stocks of Mulligan's strain were distributed among labs worldwide, and a proliferation of alternate strain names appeared in the literature. Initially it was referred to as the 'Rockefeller' strain (Garnham, 1959), or the 'TC' ('typical cynomolgi') strain (Eyles, 1960). In 1961 it as designated the 'M' strain in honor of Mulligan (Coatney et al., 1961; Schmidt et al., 1961), though it has also been referred to simply as 'Mulligan' (Cochrane et al., 1985). Adding further confusion, it was also designated as the 'neotype' strain of *P. cynomolgi* and given subspecies status as *P. cynomolgi cynomolgi* (Eyles et al., 1963). Meanwhile *P. cynomolgi bastianellii* came to be called the B strain (Contacos et al., 1962), because malariologists at the National Institutes of Health did not think that the differences between it and the neotype strain were significant enough to warrant subspecies status. Later, a line of the B strain was termed the 'NIH' strain and also

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