Note

Compensatory Evolution in the Human Malaria Parasite *Plasmodium ovale*

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ABSTRACT

The fixation of neutral compensatory mutations in a population depends on the effective population size of the species, which can fluctuate dramatically within a few generations, the mutation rate, and the selection intensity associated with the individual mutations. We observe compensatory mutations and intermediate states in populations of the malaria parasite *Plasmodium ovale*. The appearance of compensatory mutations and intermediate states in *P. ovale* raises interesting questions about population structure that could have considerable impact on the control of the associated disease.

THE probability of compensatory genetic change occurring in a gene is very small. The rate of fixation of compensatory mutations in a population depends upon three major factors: (1) effective population size, (2) selection pressure, and (3) mutation rate (Kimura 1990; Stephan 1996). Compensatory evolution has been studied extensively in relation to the RNA structure (Stephan and Kirby 1993; Kirby *et al.* 1995; Muse 1995; Stephan 1996; Innan and Stephan 2001). Maintaining the structure of ribosomal RNA (rRNA) is essential to the integrity of the molecule. Mutation in a stem, which leads to a loss of base pairing, will reduce its stability and this is often accompanied by changes in the biochemical properties of the molecule. A second mutation in the complementary region of the RNA that restores the structural integrity most often restores the biochemical properties of the molecule despite changes in the primary sequence (Figure 1A). In essence, the probability of both members of a stem pair changing in congruence as well as fixing in a population is extremely small. The compensatory changes that occur in the stem structures of the hypervariable regions in rRNA are thought to be near neutral in net effect.

We investigated the ribosomal RNA sequence from different isolates of *Plasmodium ovale* for compensatory changes and report the presence of not only compensatory mutations, but also the intermediate state in the population. The species identification of the most diverse members was confirmed by immunofluorescence assay and microscopy (Li *et al.* 1995). The variation seen here is not based on geographical separation as the isolates from both continents were used for the analysis. Both complete and partial sequences have been reported to GenBank: Nigeria (AY278222, U78740, L48986, and L48987) Ghana (AJ250701), Cameroon (X99790 and AJ001527), and Papua New Guinea (AF145337). In addition, several sequences were determined from the blood samples of recent immigrants to the Washington, DC, area who were infected with *P. ovale* and came to area hospitals for treatment (U78739, AY278223, and AY278224). The ribosomal DNA sequence from *P. simiovale* was also determined (AY278221). The sequences of the expressed RNA from our samples were verified by RT-PCR (data not shown).

Alignment of 11 available *P. ovale* sequences representing the hypervariable regions V7 and V8 of small subunit (SSU) rRNA revealed 24 phylogenetically informative positions in a 360-nucleotide segment. Maximum-parsimony analysis of all available *P. ovale* SSU rRNA distinguished four distinct clades with very high bootstrap values (Figure 1C). Even though the *P. ovale* isolates are divided into clades, they have a consistency index of >0.99, indicating a direct lineage among isolates. Hence, clades are most likely to have arisen from neighboring clades.

Next, we determined the secondary structure of *P. ovale* rRNA sequences on the basis of Robin Gutell's framework for *P. vivax* rRNA (Li *et al.* 1997), which has >90% identity in those regions and is available from the comparative RNA web site at the University of Texas (http://www.rna.icmb.utexas.edu/; Cannone *et al.* 2002).

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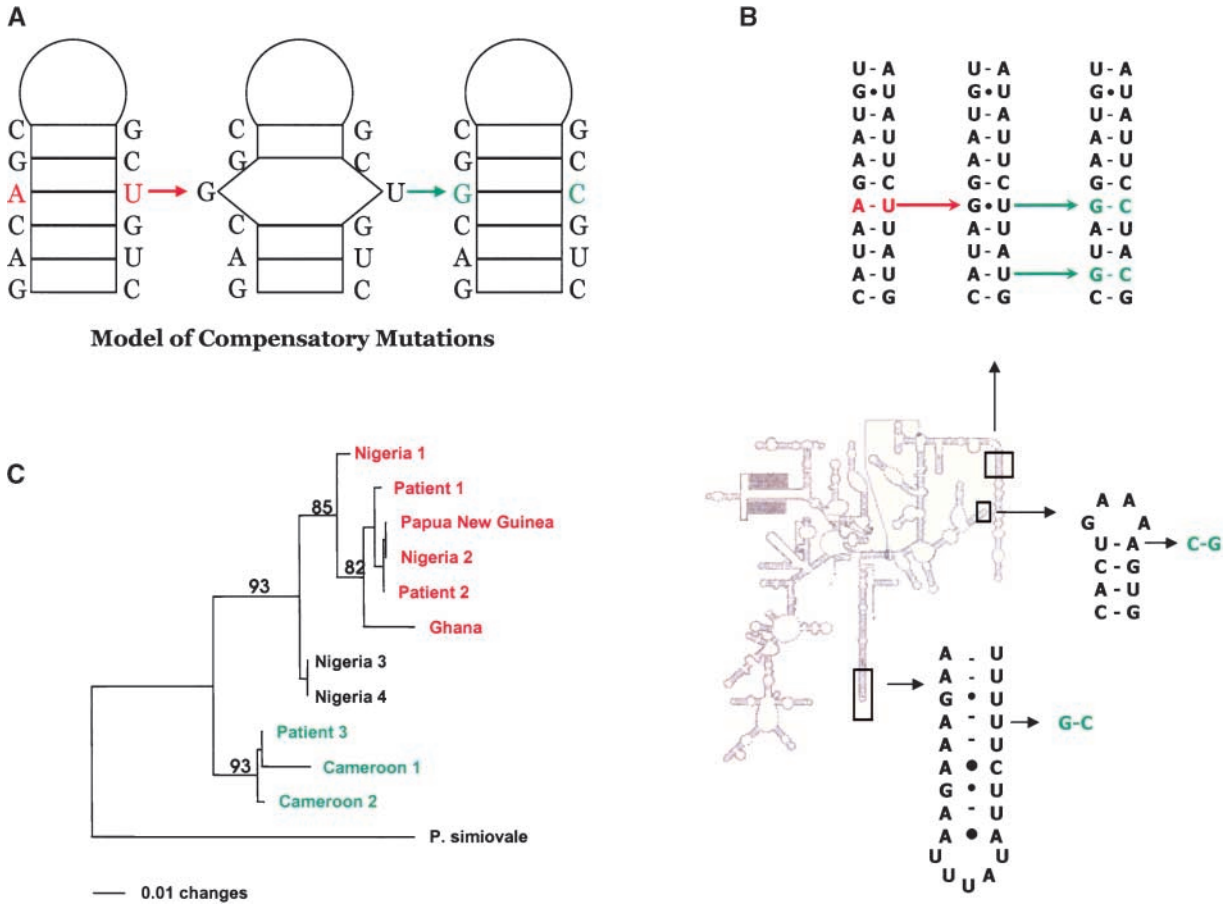

FIGURE 1.—(A) Putative sequence of events in a compensatory change (adapted from CHEN *et al.* 1999). (B) Location of compensatory changes in the V7 stem of *P. ovale.* (C) Parsimony analysis of *P. ovale* rRNA sequences from various isolates. *P. simiovale* is used as an outgroup.

On determining the secondary structure of regions V7 and V8, compensatory base-pair changes were found among the isolates (Figure 1B). Of 11 isolates, 3 (Nigeria 1, Nigeria 3, and Nigeria 4) carried the putative transition state between A-U to G-C in the form of a G•U pair at positions 1467 + 1584 (numbered according to GenBank accession no. U07367), suggesting the presence of a stable intermediate state (Figure 1B, Table 1). Three compensatory changes were found between nucleotide pairs at positions 1470–1581, 1768–1773, and 1960–1976, but their intermediates were not found (Figure 1B, Table 1).

Of the 16 possible pairing configurations, 4 Watson-Crick (G-C, C-G, U-A, and A-U) combinations represent 81% of all helical base pairs in rRNA (KONINGS and GUTELL 1995). Of the 12 remaining sets, 2 wobble pairs (G•U and U•G) represent 13% and A•G and G•A represent 3% of the total pairs (KONINGS and GUTELL 1995). Each of the other 8 possible combinations (U•U, G•G, A•C, A•A, C•C, C•U, C•A, U•C), represent <1% (KONINGS and GUTELL 1995). This selective representation is associated with the thermodynamic profile of these combinations as G•U and U•G have the highest stability among the noncanonical pairings (FREIER *et al.* 1986). The demonstration of a G•U intermediate in the mechanism of compensatory change suggests that thermostability of the intermediate state is an important factor in the transition process. All compensatory mutations in *P. ovale* follow a pathway that would be favored by the G•U pair and, in fact, the G•U intermediate is captured in three sequences from Nigeria.

Compensatory mutations were found in both stable and hypervariable regions of the RNA. Previously, SCHAEFFER and MILLER (1993) studied intraspecies polymorphism in the alcohol dehydrogenase region of *Drosophila pseudoobscura* and found that the polymorphisms in introns show significant linkage disequilibrium. Subsequently, KIRBY *et al.* (1995) investigated the secondary structure of the introns and proposed a mechanism for compensatory evolution. Although compensatory changes reported in our study follow the proposed mechanism, the finding of an intermediate is unexpected on the basis of modeling studies of populations without a substructure (INNAN and STEPHAN 2001). We propose that we find intermediate states of compensatory change in *P. ovale* rRNA because the population is splintered into

TABLE 1

Phylogenetically informative changes in the small subunit ribosomal RNA of *P. ovale* isolates

Source	Accession no.	1460	1467	1470	1471	1472	1487	1494	1511	1513	1526	1527	1542	1547	1558
Nigeria 1	AY278222	T	T	T	G	T	C	T	C	T	A	T	A	A	A
Patient 1	AY278224	T	T	T	G	T	C	G	C	T	A	T	A	T	A
PNG	AF145337	C	T	T	G	C	A	G	C	C	A	T	A	T	A
Nigeria 2	U78740	C	T	T	G	C	A	G	C	C	A	T	A	T	A
Patient 2	AY278223	C	T	T	G	C	A	G	C	T	A	T	A	T	A
Ghana	AJ250701	A	T	T	G	C	C	G	C	T	A	T	A	T	A
Nigeria 3	L48986	T	T	T	G	T	C	T	C	T	A	T	A	A	A
Nigeria 4	L48987	T	T	T	G	T	C	T	C	T	A	T	A	A	A
Patient 3	U78739	T	C	C	T	T	C	G	T	T	T	A	T	A	T
Cameroon 1	AJ001527	T	C	C	T	T	C	G	T	T	T	A	T	A	T
Cameroon 2	X99790	T	C	C	T	T	C	G	T	T	T	A	T	A	T
P. simiovale	AY278221	T	T	C	G	T	T	G	T	T	C	A	T	A	T
P. vivax	U07367	C	T	C	G	T	T	G	T	T	C	A	T	A	T

Source	Accession no.	1574	1580	1581	1583	1584	1650	1744	1746	1762	1768	1769	1773	1960	1976
Nigeria 1	AY278222	C	C	A	A	G	A	A	G	T		T			
Patient 1	AY278224	C	C	A	A	A	A	A	G	T		T			
PNG	AF145337	C	C	A	T	A	A	A	G	T		T			
Nigeria 2	U78740	C	C	A	T	A	A	A	G	T		T			
Patient 2	AY278223	C	C	A	T	A	A	A	G	T		T			
Ghana	AJ250701	C	C	A	T	A	A	A	G	T		T			
Nigeria 3	L48986	C	C	A	A	G	T	A	G	T	T	T	A	A	T
Nigeria 4	L48987	C	C	A	A	G	T	A	G	T	T	T	A	A	T
Patient 3	U78739	T	T	G	A	G	A	T	A	C		A			
Cameroon 1	AJ001527	T	T	G	A	G	A	T	A	C	C	A	G	G	C
Cameroon 2	X99790	T	T	G	A	G	A	T	A	C	C	A	G	G	C
P. simiovale	AY278221	T	C	G	A	A	A	T	C	C		G			
P. vivax	U07367	T	C	G	A	A	A	T	C	C	T	G	A	G	C

The individual nucleotides have been numbered using the *P. vivax* 18S rRNA sequence as anchor (U07367). The residue pair 1467 + 1584 in sequences from Nigeria 1, Nigeria 3, and Nigeria 4 show an intermediate state (GU) of the AU to GC compensatory change. Pairs at positions 1470 + 1581, 1768 + 1773, and 1960 + 1976 show compensatory changes without intermediate states.

a large number of genetically isolated subpopulations and undergoes frequent bottlenecks, leading to relaxation of selection pressures.

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