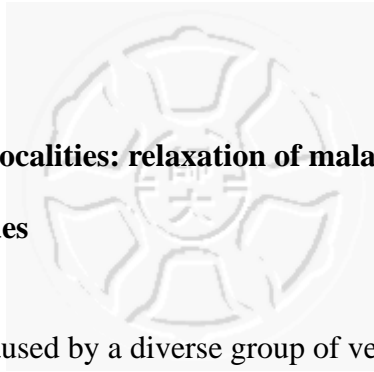


## Results

### Malarial prevalence over localities: relaxation of malarial pressure in populations at high altitudes



Since malaria is caused by a diverse group of vector-transmitted parasites that infect red blood cells, in this study, I only screened individuals (N = 139) for which blood samples were available. Twenty blood samples out of a total of 139 (14.4%) exhibited infection by *Haemoproteus spp.* (Fig. 2), and prevalence varied among altitudinal populations (low: 10.7 %; intermediate: 30.4 % and high: 0 %) (Fig.3; Table 3), indicating divergent malarial selection among altitudes.

However, malarial prevalences in the same altitudinal populations also differed significantly expect that at high altitude (LRT, two-tailed, for low altitude:  $G=11.205$ ,  $p= 0.0037$ ; for intermediate altitude:  $G=19.912$ ,  $p< 0.0001$ ). At both low and intermediate altitude, there was one population in which no malarial infection was detected. Altogether, the prevalence of malarial infection in populations of intermediate altitudes was statistically higher than in populations at lower or higher altitudes (LRT, two-tailed:  $G=19.853$ ,  $p< 0.0001$ ).

### Genetic variation of the MHC and microsatellites

All 165 individuals of the gray-cheeked fulvetta were screened for MHC and microsatellite polymorphisms (see Appendix). A total of 28 MHC class I alleles were identified using the CE-CCSP method. On average, each individual had 7.81 MHC alleles (range: 2 to 14 alleles). The proportion of these MHC alleles in different populations ranged from 3.64% to 93.94% (Table 4). All MHC class I alleles, except

*Almo*\*28, were shared across all three altitudes (Fig. 4a). Generally, individuals from three altitudinal populations carried a similar number of MHC class I alleles: averaged 8.0, 7.8 and 7.8 MHC alleles for the low, intermediate, and high altitudinal populations respectively (Fig. 5a, Kruskal-Wallis test,  $p=0.9974$ ). APD values within populations ranged from 37.69% at Nansi to 47.10% at Yufong.

A total of 82 alleles were identified for five microsatellite loci. The total number of alleles for each locus ranged from 8 to 33, with an average of 18.4. All pairs of loci were in linkage equilibrium across all populations. Individuals at different altitudes were shown to carry a significantly different number of alleles (mean  $\pm$  SE; low altitude:  $9.13 \pm 0.12$ ,  $N=56$ ; intermediate altitude:  $8.85 \pm 0.15$ ,  $N=46$ ; high altitude:  $8.73 \pm 0.11$ ,  $N=63$ , Kruskal-Wallis test,  $p= 0.022$ , Fig. 5b). Sixty-nine, 64 and 65 alleles were found in the low, intermediate and high altitudinal population, respectively (Fig. 4b). There are substantially high levels of variation within populations with mean APD values ranging from 68.10% at Rueiyan to 78.73% at Jiji.

The patterns of genetic variation at MHC and microsatellites over three altitudinal populations were different since that no significant correlation was found between MHC diversity and microsatellite heterozygosity per individual (Fig. 6;  $\rho = -0.0319$ ,  $p=0.682344$ ,  $N=165$ ), indicating that there was no significant influence of genome-wide variability on MHC diversity. In addition, I found that APD values for MHC data were not significantly correlated with those of microsatellites from the same populations ( $r = -0.27011$ ,  $p= 0.4821$ , see Fig. 7), suggesting that diversity at microsatellites and MHC loci may be driven by different evolutionary forces.

## **Association between specific MHC alleles and divergent malarial pressure across altitudes**

I define localities at low and intermediate altitudes as the endemic region, and those at high altitudes as the non-endemic region. In endemic region, MHC allele *Almo\*05* was identified as explaining a significant proportion of malarial infection (forward stepwise regression analysis,  $p=0.0062$ ,  $\log(p/1-p)=6.38+0.78 \times Almo*05$ , odds ratio=4.78). This was also true if stepwise regression was implemented using data of four endemic localities at low and intermediate altitudes only (forward stepwise regression analysis,  $p=0.0034$ ,  $\log(p/1-p)=0.40+1.00 \times Almo*05$ , odds ratio=7.48). Furthermore, individuals carrying the *Almo\*05* allele were significantly more likely to be infected with malaria (Fisher's exact test, one-tailed:  $p=0.0052$  in the endemic region;  $p=0.0091$  in the four endemic localities, respectively). In addition, I found frequency of MHC allele *Almo\*05* to be significantly higher in populations at the non endemic region than the endemic one (Fisher's exact test, one-tailed:  $p=0.0287$ ), but not significantly different in populations at endemic localities. In my study, odds ratio was ranged from 0.28 to 7.06 for 28 MHC alleles, implying that MHC alleles may confer resistance or susceptibility to malarial infection. However, only *Almo\*05* showed significant effect in stepwise logistic regression test.

## **Balancing selection on MHC polymorphism in all populations**

I compared the differentiation of allele frequencies in the three altitudinal populations at micosatellites and MHC as measured by  $F_{ST}$  values from the AMOVA analysis. Frequency of microsatellite alleles was slightly but significantly different between three altitudinal populations (82 microsatellite alleles, AMOVA<sub>3,164</sub>,  $F_{ST}=0.00057$ ,  $p=0.04692$ ), whereas that of MHC alleles was not (28 MHC alleles,

AMOVA<sub>3,164</sub>,  $F_{ST} = -0.00032$ ,  $p = 0.45064$ ). The observation of a more even allelic distribution at MHC than that at microsatellite loci was concordant with the expectation of balancing selection shaping MHC diversity.