Discussion

Here I present the evidences to support the pathogenic selection gradients along altitudes shaping the diversity and allelic distribution of MHC alleles in the gray-cheeked fulvetta. Using the gray-cheeked fulvetta populations and malaria as a model, specific MHC allele conferring susceptibility to malaria was found and its allele frequency distribution showed altitudinal trend, possibly underlined by varying intensity of malarial pressure. This study demonstrates that specific MHC allele may be subjected to divergent selection over a fine spatial scale, while on the other hand balancing selection may play a key role in maintaining MHC polymorphism in the gray-cheeked fulvetta populations across different altitudes.

Divergent altitudinal malarial pressures in the gray-cheeked fulvetta populations

The decreased temperature due to increased altitude not only limits the distribution (Epstein 2001) and population dynamics of vectors at high altitudes, but also slows the developmental rate of malarial parasites in their vectors and lowers the frequency of vectors infected by malaria (Woodworth *et al.* 2005), thereby decreasing malarial pressures on hosts. However, in this study, I found malarial prevalence in the intermediate altitudinal populations to be higher than that in the low altitudinal populations. This phenomenon may be mainly attributed to high prevalence of malarial infection of Aowanda population at the intermediate altitudes (11/17, 64.7 %). In addition, malarial prevalence in low and intermediate altitudinal localities was significantly different, showing high level of heterogeneity of malarial pressure. Therefore, lower malarial prevalence in the low altitudinal populations might be an artifact of sampling variation. Such a confounding pattern of malarial prevalence along altitudes resulting from within-sample variation in altitudinal malarial

endemicity has also been found in another study (Cattani *et al.* 1986). Although the density of the malaria vectors is still unknown, PCR-based diagnosis in this study suggested the absence of malarial pressures at high-altitude, and populations at low and intermediate altitudes were under stronger malarial selection pressure. Such altitudinal divergent selection should facilitate the differentiation of MHC genes among local gray-cheeked fulvetta populations.

Differentiation of specific MHC class I allele frequency under divergent malarial pressures

Specific MHC alleles may be of different fitness consequences which are context dependent. Some MHC alleles can confer susceptibility to infectious and autoimmune disease, but some confer resistance. Theoretically, MHC alleles susceptible to malaria may be selected against in environments with high level of malarial pressure may select against, but persist in environments with low malarial prevalence. Indeed, I found individuals carrying the common allele *Almo**05 to have increased susceptibility to malarial infection, and *Almo**05 was of significantly higher frequency at high altitudes than at low and intermediate altitudes. Such observation implied that higher malarial pressures at low altitudes selects for decreasing frequency of the *Almo**05 allele, but as malarial pressure relaxes towards higher altitudes, frequency of *Almo**05 frequency become more so determined by drift.

Given the few empirical observations, it is nevertheless difficult to predict pattern MHC variation within various environmental contexts. Therefore, whatever adaptive pattern of MHC variation revealed in this study pertains only to malarial infection, but one could easily extend this conclusion and imagine other pathogenic pressures shaping the frequency distributions of various MHC alleles.

Frequency of MHC allele conferring susceptibility

Many susceptible MHC alleles have been found in studies of immune recognition, but the fact that such deleterious alleles persist in populations is still controversial. McClelland (McClelland *et al.* 2003) provided the first experimental evidence that heterozygote superiority emerged when resistance alleles are dominant, that is, resistance allele would mask the effect of susceptible allele in heterozygotes. The mechanism is suggested to be contributed to the evolution of MHC diversity and is an explanation of existence of susceptible alleles. In my work, a susceptible MHC allele *Almo**05 was found to be of high frequency. Although my study lacks information of diseases other than malaria and susceptibility profile of MHC alleles, the phenomena that gray-cheeked fulvetta populations are under multiple disease challenges and there is dominance effect of specific alleles are commonly observed (Behnke & Wahid 1991; Chen *et al.* 1992), thereby providing a plausible explanation for *Almo**05 to be retained and increase in frequency. Nevertheless, a non-exclusive explanation remains that high frequency of susceptible MHC allele is a reflection of immune trade-off. Since selection favors MHC molecules which can present an increased spectrum of pathogens, peptide binding sites of these molecules would be kept constant to avoid changing their function (Meyer & Thomson 2001). However, some pathogens presented by the specific MHC molecules may evolve successfully to evade detection (de Campos-Lima *et al.* 1993), in such cases MHC molecule resistant to some pathogens may be susceptible to others, thus allelic frequency becomes a function of interaction between numerous pathogenic peptides and MHC molecules (McClelland et al. 2003). Lastly, fitness consequences of *Haemoproteus* on birds remains controversial (Kleindorfer *et al.* 2006; Marzal *et al.* 2005; Rätti *et al.* 1993; Ruiz *et al.* 1995), and according to field inspection, infected individuals did not show

symptoms of illness, implying that *Haemoproteus spp.* results in little fitness reduction, hence MHC allele *Almo**05 may be subjected to weak selection. To confirm this inference, future investigation is needed to identify various associations between sequences or even tertiary structures of MHC alleles and multiple pathogen peptides.

Balancing selection across the three altitudinal habitats maintains overall polymorphism of MHC class I genes

Contrasting the partitioning of genetic variation at both neutral markers and at the MHC genes between populations can yield insight into the spatial heterogeneity of selective pressures (as reviewed by Garrigan & Hedrick 2003). If variation in pathogenic constitution and virulence exert differential selection among populations, allele frequency distribution should be more dissimilar at the MHC genes than at microsatellites (Beaumont 2005). In contrast to the expectation of such pathogen-mediated directional selection, my study showed that in general MHC allele frequency was not significantly differentiated between altitudinal populations, whereas variation of microsatellites was slightly but significantly differentiated. This pattern was concordant to the expectation of pathogen-mediated balancing selection, whereby environments across altitudes all exerted high pathogenic selection. Because the number of MHC loci is limited and each MHC allelic product only recognizes a limited array of foreign peptides (Edwards et al. 2000), to face the tremendous pathogenic selection in the different environments, MHC diversity are more likely to be maintained across the altitudinal gradient. Moreover, although the pathogenic fauna in the different altitudes could be drastically different, the pleiotrophic effect of MHC allelic product (Hughes 1999) could also maintain the MHC diversity in

different altitudinal populations. Alternatively, MHC alleles under balancing selection across altitudes may be of higher effective migration rate than neutral alleles. In conclusion, the total number of MHC alleles under balancing selection was less affected by population structure, but specific allele may be under divergent altitudinal selection. My result was consistent with that found in the guppy (*Poecilia reticulate*) of Trinidad, where populations from upper and lower Aripo river were much less differentiated at MHC genes than at microsatellites (Van Oosterhout *et al.* 2006).