Introduction

Local adaptation refers to the process of differentiation among populations facing different selective regimes (Williams 1966). Classic examples such as the color of peppered moths, *Biston betularia* (see Rudge 1999), tolerance for heavy metals in plants (Macnair 1987), and the beak sizes of *Geospiza* finches (Grant *et al.* 1976) have aptly illustrated how populations adapted to strong local selective agents. In some cases, the strength of local adaptation even outweighs the homogenizing effect of inter-population gene flow (reviewed by Kawecki & Ebert 2004; Rundle & Nosil 2005), thereby allowing species to diverge non-allopatrically (Dieckmann *et al.* 2004). Owing to the central role of local adaptation in generating biodiversity, elucidating the mechanism of local adaptation has been a ceaseless pursuit in evolutionary biology.

Local adaptation occurs when there exist heterogeneous selective pressure across landscapes, involving nonbiological (*e.g.* temperature, soil type…etc.) and biological factors (*e.g.* competitors and parasitic fauna…etc.), which taken together often constitute spatial gradients. Differentiation of allele or character frequency under such environmental gradients, often latitudinal, have extensively been documented in plants, *Drosophila*, and vertebrates (*e.g.* Land *et al.* 1999; Oleksyn *et al.* 1998; Palo *et al.* 2003). Such gradients are potentially mirrored and compacted along altitudes, however, relatively fewer studies (*e.g.* Ledig & Korbobo 1983; Miaud & Merilä 2000) have revealed corresponding adaptation.

Among environmental factors, selection driven by certain pathogens might show a strong altitudinal gradient. Pathogens represent a powerful selective agent in their natural host populations by incurring mortality (as reviewed by Altizer *et al.* 2003) and potentially, as results of both biotic and abiotic factors, environments along

altitudes may vary in the selection intensity of specific pathogen accordingly and harbour heterogeneous pathogens that impose different selective pressures. A notable example of such phenomenon is malarial infection. Malaria is a haematozoan disease caused by five genera of blood parasites (*Haemoproteus, Plasmodium, Hepatozoon, Tyrpanosoma* and *Leucocytozoon*). Among which *Haemoproteus* and *Plasmodium* parasites are known to be transmitted by free-flying dipterans, especially mosquitoes, whose distribution is restricted to places of higher temperature and humidity (Molineaux & Gramiccia 1980; Onori & Grab 1980). It was evidenced that the prevalence of malaria decreases predictably with the abundance of vectors (Bynum 1999; Hay *et al.* 2004), especially mosquitoes, with increasing altitudes: in Hawaii, the introduced mosquitoes transmitted *Plasmodium relictum* to the Hawaii Amakihi (*Hernignathus virens*), causing populations of this avian species to decline dramatically in lowland (Warner 1968); in Thailand, more human individuals in lowland got malarial infection than those in highland (Wiwanitkit 2006). Consequently, studying the association of malarial pressures and corresponding defense mechanism in the host populations from different altitudes provides a platform to study altitudinal adaptation.

Malaria imposes stress on the host's immune system, whose responses included marked contribution of the major histocompatibility complex (MHC) genes (as reviewed by Hill *et al.* 1997). The MHC genes encode molecules responsible for recognizing self-nonself antigen fragments and presenting foreign peptides to T cells, thereby triggering adaptive immune responses (see Edwards *et al.* 2000). Indeed, associations between malarial resistance and specific MHC alleles or allelic diversity have been found (*e.g.* in human, Hill 1991, and in birds, Bonneaud *et al.* 2006; Westerdahl *et al.* 2005). On the other hand, positive correlation may also exist

between specific MHC alleles and malarial infection, such as the finding that MHC allele B4b of great reed warbler (*Acrocephalus arundinaceus*) was linked to increased susceptibility to malaria GRW2 parasitic infection (Westerdahl et al. 2005). Because MHC alleles exhibit a tight correlation with malaria, geographically different malarial pressures may be prominent in determining the frequencies of particular MHC alleles.

The adaptation of host resistance traits in response to pathogens has been supported by the observed associations between resistance frequency and levels of parasite prevalence in natural populations (Burdon & Thrall 1999; Lively 1992). However, local adaptation requires any difference to be of a heritable basis and yet, except studies in some model species (Prugnolle *et al.* 2005; Wegner *et al.* 2003), there has been little direct evidence demonstrating the association between patterns of genetic variation and geographically heterogeneous pathogenic selection (Ekblom *et al.* 2007; Miller *et al.* 2001). Here I used the gray-cheeked fulvetta (*Alcippe morrisonia morrisonia*) in different altitudes of Taiwan and the malarial prevalence in each population to study how altitudinal pathogenic pressure may shape the diversity of fitness-related, hence adaptation-prone, the MHC class I genes, which are expressed on nearly all nucleated cells (including avian red blood cells) and responsible for recognizing and presenting intracellular pathogens such as viruses and protozoans (*e.g. Plasmodium* and *Haemoproteus*), were examined. The gray-cheeked fulvetta is a small-sized passerine that is commonly distributed in the margins of the forest from zero to 2800 m above sea level in Taiwan, an island situated in the subtropical and tropical eastern Asia. Banding records (Yao, personal communication) suggest that the birds do not appear to have seasonal vertical migration, i.e. are relatively sedentary, such that populations at different altitudes may face varying malarial pressures. Consequently, the immune system of the gray-cheeked fulvetta

may have faced and co-evolved with malarial pressure in local environments to which they are exposed.

Upon demonstrating that divergent selection pressure existed among altitudinal populations, the following questions were then addressed: 1) was there any association between specific MHC alleles and malarial infection in gray-cheeked fulvetta populations with high malarial prevalence? 2) Did frequencies of these specific MHC alleles differentiate corresponding to malarial pressure along the altitudes? In addition, I was interested in knowing which evolutionary force maintained overall MHC polymorphism among altitudes. To avoid the confounding effects of demography on MHC diversity, variation of microsatellites was employed to serve as neutral control against which the relative role of selection in shaping MHC variation could be inferred (Garrigan & Hedrick 2003).