# ERRATA

# GENETIC DIFFERENTATION AMONG THREE POPULATIONS OF *ANOPHELES MINIMUS* OF GUANGXI AND YUNNAN PROVINCES IN THE PEOPLE'S REPUBLIC OF CHINA

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**Abstract.** Electrophoretic studies were carried out on isozymes of 3 populations of *Anopheles minimus* collected from Guangxi and Yunnan Provinces of the People's Republic of China in 1993. Eight proteins were analyzed by 5% polyacrylamide gel electrophoresis. The most variable population, 'Yunnan-Field' (Y-F), was highly polymorphic at 14 of 20 loci ($P = 0.700$) with an average heterozygosity $\bar{H}$ of 0.340. $P$ values of 0.500 and 0.700, and $\bar{H}$ values of 0.220 and 0.210 were obtained for each from 'Guangxi-Lab' (GX-L) and 'Yunnan-Lab' (Y-L), respectively, Nei's genetic distances ($D$) between Y-L and GX-L, Y-F and GX-L, and Y-F and Y-L were 0.1131, 0.1946 and 0.1069, respectively. These results suggest that GX-L is distant from the 2 other populations, Y-L and Y-F, and that this genetic differentiation between the 2 populations of Yunnan and Guangxi Provinces corresponds to the forms A and B, which were morphologically classified by Xu *et al* (unpublished).

## INTRODUCTION

In China, malaria cases are numerous in rural areas, especially south of 25° NL (Nanling mountain), which includes the hyper- and meso-endemic regions of Guangxi and Yunnan Provinces, where falciparum malaria is frequent (Tang *et al*, 1991). Among the 7 major malaria vectors in China, *An. minimus, An. jeyporiensis candidiensis* and *An. fluviatilis* are the main vectors south of 25° NL (Ren *et al*, 1984; Deng, 1980).

*Anopheles minimus* of China has morphological and ecological varieties (Ho, 1938; Liu *et al*, 1959; Yu and Li, 1984). These variations provide impetus to epidemiologic analysis of malaria, therefore genetic study of the vectors is essential. Additionally, the distribution of many animals and insects is now gradually changing because of the increase in global atmospheric temperature. Characteristics of the various species of malaria vector mosquitos may also be changing, and more details of their genetic background should be studied.

Some genetic analysis using protein electrophoresis in *An. minimus* were performed in Thailand (Sucharit *et al*, 1988; Komalamisra, 1989), but have not previously been made in China. In the present study, we carried out an isozyme analysis of 3 populations from Guangxi and Yunnan Provinces of the People's Republic of China.

## MATERIALS AND METHODS

### Mosquito populations used

Three populations of *An. minimus* were obtained from Guangxi and Yunnan Provinces. In August 1993, females of the strain designated 'Yunnan-Field' (Y-F) were collected from human dwellings in the suburbs of Simao, Yunnan Province. These females were individually reared in glass tubes for oviposition. After the hatching of eggs, larvae were reared under an 8L-16D photoregine at 24 ± 1°C and 80% relative humidity with an artificial diet (*ca* 2.0 mg/day).

Two laboratory populations, designated 'Guangxi-Lab' (GX-L) and 'Yunnan-Lab' (Y-L) were originally collected in the suburbs of Baise, Guangxi Province in 1993, and in the suburbs of Simao, Yunnan Province in 1992, respectively.

They were colonized at the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, as was the Y-F population. At the end of the 4th-instar, larvae of both populations were put into microtubes (1.8 ml Eppendorf tubes) and stored at -80°C until electrophoresis.

**Sample preparation for electrophoresis**

Larvae were individually homogenized in 10-15 $\mu$l of 5 mM 2-mercaptoethanol using a Teflon homogenizer in an ice-bath. Ten $\mu$l of 5 mM 2-mercaptoethanol was used for detection of octanol dehydrogenase (Odh), aldehyde oxidase (Aox) and alkaline phosphatase (Alkp). For general protein (Gp5), lactate dehydrogenase (Ldh), 2 carboxyl esterases (Est-$\alpha$ and Est-$\beta$) and aminopeptidase (Lap), 15 $\mu$l of 5 mM 2-mercaptoethanol were used. Homogenates were then centrifuged at 10,000 *g* for 10 minutes at 4 °C, and 5 $\mu$l of the supernatant fluid was loaded into the sample slot.

**Electrophoresis and calculation of genetic diversity**

Electrophoresis was performed in a 5% horizontal polyacrylamide gel at 4°C. Electrophoretic techniques usually followed the method of Tsukamoto (1984, 1989). Buffer systems for proteins examined are shown in Table 1. Eight proteins from the 3 populations of *An. minimus* provided discrete bands and were selected for characterization. Twenty (GX-L, Y-L) or 30 larvae (Y-F) were used in each protein electrophoresis.

After the gel film had dried, the migration distance of each band was measured and the relative mobility was calculated as the $R_f$ value (×100). The genetic variability in a population was quantified by measuring the proportion of polymorphic loci ($P$) and the average heterozygosity per individual ($H$). The average heterozygosity is calculated as

$$\bar{H} = 1 - \overline{\Sigma q_i^2},$$

when $q_i$ is the frequency of the i-th allele at a locus, and the average was calculated for all 20 loci examined. The intraspecific gene differences among the 3 populations were estimated by calculating Nei's genetic distance ($D$) and genetic identity ($I$) (Nei, 1972).

## RESULTS

**Description of protein variants**

Fig 1 shows band morph patterns of the 3

Table 1

Proteins examined in electrophoretic analysis of 3 populations of *Anopheles minimus*.

| Proteins | Abbreviation | Enzyme commision no. | Gel buffer |
|---|---|---|---|
| Alkaline phosphatase | Alkp | EC 3.1.3.1 | 0.1 M Tris-HCl, pH9.5 |
| Aldehyde oxidase | Aox | EC 1.2.3.1 | 0.1 M Tris-HCl, pH7.4 |
| Carboxylesterase-α | Est-α | EC 3.1.1.1 | 0.2 M Phosphate, pH7.4 |
| Carboxylesterase-β | Est-β | EC 3.1.1.1 | 0.2 M Phosphate, pH7.4 |
| Leucine aminopeptidase | Lap | EC 3.4.11.1 | 0.2M Phosphate, pH7.4 |
| Lactate dehydrogenase | Ldh | EC 1.1.1.27 | 0.1 M Tris-HCl, pH7.4 |
| Octanol dehydrogenase | Odh | EC 1.1.1.73 | 0.1 M Tris-HCl, pH8.5 |
| General protien | Gp5 | | |

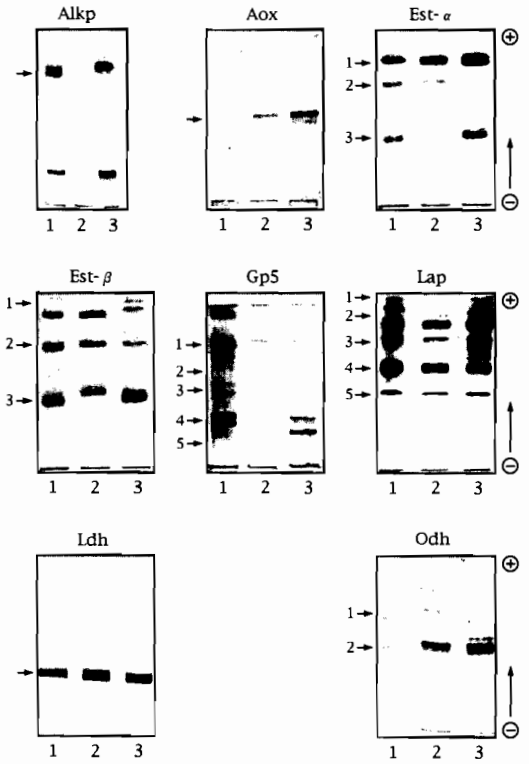


Fig 1–Band morph patterns of the 3 populations of Anopheles minimus at 8 protein loci. A single larva was loaded on each lane. 1, GX-L; 2, Y-L; 3, Y-F.

populations. Of the 20 protein loci examined, 3 loci, namely Gp5-2, Gp5-5 and Lap-4, showed no variation among the 3 populations. The allele frequency and genotype frequency variants of each protein are shown in Table 2 and Table 3, respectively. Electrophoretic patterns were examined for their goodness-of-fit with regard to expectations based on the Hardy-Weinberg equilibrium (HWE) model. Chi-square ($\chi^2$)-tests to fit the HWE showed that the distribution of the genotypes of almost all the polymorphic proteins examined (Table 3) were not significantly outside HWE. Detailed interpretations of protein variants are as follows:

**Alkaline phosphatase (Alkp):** One locus appeared in each population. In GX-L and Y-L, the *Alkp*$^{84}$ allele was dominant (both allele frequencies were 0.950) (Table 3), indicating a homozygote, but a few individuals showed heterozygosity constructed by *Alkp*$^{84}$ and *Alkp*$^{82}$ (Table 2). The *Alkp*$^{84}$ allele was also dominant in Y-F (allele frequency was 0.483), but this locus was found to be highly polymorphic and possessed 4 alleles (Table 3).

**Aldehyde oxidase (Aox):** The Aox locus showed high polymorphism in all populations examined, where the most common allele, *Aox*$^{44}$, had a value of 0.500, 0.825 and 0.483 in GX-L, Y-L and Y-F, respectively (Table 3). The highest polymorphic level had 5 alleles in Y-F.

**Carboxylesterase-α (Est-α):** At the loci Est-α-1 and Est-α-2, GX-L had only one allele, *Est-α-1*$^{84}$ and *Est-α-2*$^{68}$, respectively, while Y-L and Y-F showed high polymorphism (Table 3). Especially in Y-F, 6 and 4 genotypes in locus Est-α-1 and Est-α-2, respectively, were found to have been constructed by 4 alleles (Table 2). In the Est-α-3 locus, each population had 2 alleles, *Est-α-3*$^{44}$ and *Est-α-3*$^{40}$; the former was dominant in GX-L, while the latter was dominant in Y-L and Y- F.

**Carboxylesterase-β (Est-β):** Three loci appeared in all populations. Each one showed polymorphism, but Est-β-1 (0.542) and Est-β-3 (0.627) showd a higher allele frequency in Y-F. In locus Est-β-1, *Est-β-1*$^{94}$ and *Est-β-1*$^{90}$ were common in each population. The allele *Est-β-1*$^{84}$ was found in Y-L, but *Est-β-1*$^{92}$ was found in Y-F. In locus Est-β-2, *Est-β-2*$^{70}$ was dominant in GX-L and Y-L, while *Est-β-2*$^{72}$ was dominant in Y-F. Two alleles in both GX-L and Y-L, and 3 alleles in Y-F were found in locus Est-β-3.

**General protein (Gp5):** Five protein loci appeared in all populations. Two of the 5, Gp5-2 and Gp5-5, each had one allele, *Gp5-2*$^{72}$ and Gp5-5$^{27}$, respectively. These 2 protein loci appeared to be monomorphic loci in the present analysis. In the Gp5-1 locus, GX-L and Y-F indicated homozygote, *Gp5-1*$^{82}$, and in Y-L, this allele was also highly predominant (0.975). In the Gp5-3 locus, the *Gp5-3*$^{54}$ and *Gp5-3*$^{51}$ alleles appeared heterozygous, and *Gp5-3*$^{54}$ was dominant; allele frequency was 0.605, 0.8 and 0.983 in GX-L, Y-L and Y-F, respectively. The Gp5-4 locus was found to be polymorphic and possessed 3 alleles in all populations, however, the *Gp5-4*$^{32}$ allele was not found in GX-L and Y-L and *GP5-4*$^{40}$ was not found in Y-F.

**Leucine aminopeptidase (Lap):** Five protein loci appeared in all populations. The locus Lap-1 was clearly detected on the gel but was obtained from only a few samples in each population. Therefore, the data on locus Lap-1 was omitted. The

Table 2

Genetic frequencies at 17 polymorphic protein loci in 3 populations of *Anopheles minimus*; 3 other loci were monomorphic.

| Locus and genotype | GX-L | Y-L | Y-F |
|---|---|---|---|
| Alkp | | | |
| *85/85* | 0* (0.0)** | 0 (0.0) | 7 (2.4) |
| *85/82* | 0 (0.0) | 0 (0.0) | 3 (3.4) |
| *84/84* | 18 (18.1) | 18 (18.1) | 10 (7.0) |
| *84/82* | 2 (1.9) | 2 (1.9) | 9 (5.8) |
| *83/83* | 0 (0.0) | 0 (0.0) | 1 (0.0) |
| n | 20 | 20 | 30 |
| Aox | | | |
| *46/46* | 0 (0.0) | 1 (0.1) | 2 (1.0) |
| *46/44* | 1 (0.5) | 0 (0.0) | 0 (5.3) |
| *46/40* | 0 (0.2) | 0 (0.0) | 7 (3.3) |
| *44/44* | 1 (5.0) | 14 (13.6) | 9 (7.0) |
| *44/40* | 7 (4.0) | 0 (0.0) | 11 (8.7) |
| *44/36* | 10 (5.5) | 5 (4.1) | 0 (0.5) |
| *40/36* | 1 (2.2) | 0 (0.0) | 0 (0.3) |
| *36/30* | 0 (0.0) | 0 (0.0) | 1 (0.0) |
| n | 20 | 20 | 30 |
| Est-α-1 | | | |
| *87/84* | 0 | 0 (0.0) | 4 (2.1) |
| *87/82* | 0 | 0 (0.0) | 1 (2.3) |
| *86/86* | 0 | 0 (0.0) | 1 (0.0) |
| *84/84* | 20 | 5 (6.3) | 3 (5.0) |
| *84/82* | 0 | 10 (7.5) | 14 (11.2) |
| *82/82* | 0 | 1 (2.3) | 6 (6.3) |
| n | 20 | 16 | 29 |
| Est-α-2 | | | |
| *72/68* | 0 | 1 (0.9) | 8 (4.8) |
| *72/66* | 0 | 17 (9.0) | 11 (5.3) |
| *72/64* | 0 | 0 (0.0) | 10 (4.8) |
| *68/68* | 20 | 0 (0.1) | 1 (0.8) |
| *68/66* | 0 | 1 (1.0) | 0 (1.8) |
| *66/66* | 0 | 1 (5.0) | 0 (1.0) |
| n | 20 | 20 | 30 |
| Est-α-3 | | | |
| *44/44* | 5 (6.1) | 13 (12.8) | 16 (14.0) |
| *44/40* | 12 (9.9) | 6 (3.2) | 9 (13.0) |
| *40/40* | 3 (4.1) | 1 (0.8) | 5 (8.2) |
| n | 20 | 20 | 30 |
| Est-β-1 | | | |
| *94/94* | 3 (3.0) | 0 (0.2) | 4 (3.1) |
| *94/92* | 0 (0.0) | 0 (0.0) | 10 (11.1) |
| *94/90* | 9 (9.1) | 4 (3.4) | 1 (0.2) |
| *92/92* | 0 (0.0) | 0 (0.0) | 11 (10.0) |
| *92/90* | 0 (0.0) | 0 (0.0) | 2 (2.9) |
| *90/90* | 7 (7.0) | 15 (14.5) | 1 (0.2) |
| *84/84* | 0 (0.0) | 1 (0.1) | 0 (0.0) |
| n | 19 | 20 | 29 |
| Est-β-2 | | | |
| *72/72* | 0 (0.8) | 3 (3.2) | 12 (14.0) |
| *72/70* | 8 (6.4) | 10 (9.6) | 17 (13.0) |
| *70/70* | 12 (12.8) | 7 (7.2) | 1 (3.0) |
| n | 20 | 20 | 30 |
| Est-β-3 | | | |
| *44/44* | 2 (4.1) | 11 (15.5) | 8 (5.2) |
| *44/40* | 14 (9.9) | 9 (7.0) | 0 (4.2) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *44/38* | 0 | (0.0) | 0 | (1.0) | 5 (9.6) |
| | *40/40* | 4 | (6.1) | 0 | (0.0) | 3 (0.8) |
| | *40/38* | 0 | (0.0) | 0 | (0.0) | 4 (3.8) |
| | *38/38* | 0 | (0.0) | 0 | (0.0) | 8 (4.4) |
| | n | 20 | | 20 | | 28 |
| Gp5-1 | | | | | | |
| | *84/82* | 0 | | 1 | (1.0) | 0 |
| | *82/82* | 20 | | 19 | (19.0) | 29 |
| | n | 20 | | 20 | | 29 |
| Gp5-3 | | | | | | |
| | *54/54* | 6 | (7.0) | 13 | (12.8) | 28 (28.0) |
| | *54/51* | 11 | (9.1) | 6 | (6.4) | 1 (1.0) |
| | *51/51* | 2 | (3.0) | 1 | (0.8) | 0 (0.0) |
| | n | 19 | | 20 | | 29 |
| Gp5-4 | | | | | | |
| | *40/34* | 1 | (0.1) | 1 | (0.4) | 0 (0.0) |
| | *37/37* | 13 | (12.5) | 3 | (6.1) | 8 (8.5) |
| | *37/34* | 4 | (4.2) | 16 | (9.4) | 14 (14.4) |
| | *37/32* | 0 | (0.0) | 0 | (0.0) | 1 (0.5) |
| | *34/34* | 0 | (0.3) | 0 | (3.6) | 7 (6.3) |
| | n | 18 | | 20 | | 30 |
| Lap-2 | | | | | | |
| | *83/81* | 0 | | 2 | (1.9) | 0 |
| | *81/81* | 20 | | 18 | (18.1) | 30 |
| | n | 20 | | 20 | | 30 |
| Lap-3 | | | | | | |
| | *72/70* | 0 | | 2 | (1.9) | 0 (0.0) |
| | *70/70* | 20 | | 18 | (18.1) | 27 (27.1) |
| | *70/64* | 0 | | 0 | (0.0) | 2 (1.9) |
| | n | 20 | | 20 | | 29 |
| Lap-5 | | | | | | |
| | *46/46* | 0 | (0.1) | 0 | (0.0) | 1 (1.2) |
| | *46/41* | 2 | (0.8) | 1 | (0.8) | 10 (7.6) |
| | *44/44* | 8 | (6.6) | 3 | (0.5) | 5 (0.8) |
| | *44/41* | 7 | (8.6) | 0 | (5.0) | 0 (6.3) |
| | *41/41* | 3 | (2.8) | 16 | (13.6) | 14 (12.0) |
| | n | 20 | | 20 | | 30 |
| Ldh | | | | | | |
| | *40/40* | 0 | | 0 | | 9 (6.1) |
| | *40/36* | 0 | | 0 | | 9 (14.9) |
| | *36/36* | 20 | | 20 | | 12 (9.1) |
| | n | 20 | | 20 | | 30 |
| Odh-1 | | | | | | |
| | *61/61* | 0 | | 0 | | 1 (0.3) |
| | *61/56* | 0 | | 0 | | 4 (1.9) |
| | *59/59* | 20 | | 18 | | 12 (10.2) |
| | *59/56* | 0 | | 0 | | 11 (11.1) |
| | *56/56* | 0 | | 0 | | 2 (3.0) |
| | n | 20 | | 18 | | 30 |
| Odh-2 | | | | | | |
| | *44/44* | 1 | (5.5) | 20 | | 20 |
| | *44/40* | 13 | (6.8) | 0 | | 0 |
| | *44/38* | 6 | (3.2) | 0 | | 0 |
| | n | 20 | | 20 | | 20 |

* Observed number

** Expected number, assuming of random mating

Table 3

Allele frequencies at 20 loci including monomorphic loci in 3 populations of *Anopheles minimus*.

| Locus | Allele | GX-L | Y-L | Y-F |
|---|---|---|---|---|
| Alkp | 85 | | | 0.283 |
| | 84 | 0.950 | 0.950 | 0.483 |
| | 83 | | | 0.033 |
| | 82 | 0.050 | 0.050 | 0.200 |
| | *H** | 0.095 | 0.095 | 0.645 |
| Aox | 46 | 0.025 | 0.050 | 0.183 |
| | 44 | 0.500 | 0.825 | 0.483 |
| | 40 | 0.200 | | 0.300 |
| | 36 | 0.275 | 0.125 | 0.017 |
| | 30 | | | 0.017 |
| | *H* | 0.634 | 0.301 | 0.643 |
| Est-α-1 | 87 | | | 0.086 |
| | 86 | | | 0.034 |
| | 84 | 1.000 | 0.625 | 0.414 |
| | 82 | | 0.375 | 0.466 |
| | *H* | | 0.469 | 0.603 |
| Est-α-2 | 72 | | 0.450 | 0.483 |
| | 68 | 1.000 | 0.050 | 0.167 |
| | 66 | | 0.500 | 0.183 |
| | 64 | | | 0.167 |
| | *H* | | 0.545 | 0.677 |
| Est-α-3 | 44 | 0.550 | 0.800 | 0.683 |
| | 40 | 0.450 | 0.200 | 0.317 |
| | *H* | 0.495 | 0.320 | 0.433 |
| Est-β-1 | 94 | 0.395 | 0.100 | 0.328 |
| | 92 | | | 0.586 |
| | 90 | 0.605 | 0.850 | 0.086 |
| | 84 | | 0.050 | |
| | *H* | 0.478 | 0.265 | 0.542 |
| Est-β-2 | 72 | 0.200 | 0.400 | 0.683 |
| | 70 | 0.800 | 0.600 | 0.317 |
| | *H* | 0.320 | 0.480 | 0.433 |
| Est-β-3 | 44 | 0.450 | 0.775 | 0.431 |
| | 40 | 0.550 | 0.225 | 0.172 |
| | 38 | | | 0.397 |
| | *H* | 0.495 | 0.349 | 0.627 |
| Gp5-1 | 84 | | 0.025 | |
| | 82 | 1.000 | 0.975 | 1.000 |
| | *H* | | 0.049 | |
| Gp5-2 | 72 | 1.000 | 1.000 | 1.000 |
| Gp5-3 | 54 | 0.605 | 0.800 | 0.983 |
| | 51 | 0.395 | 0.200 | 0.017 |
| | *H* | 0.478 | 0.320 | 0.034 |

| | | | | |
|---|---|---|---|---|
| Gp5-4 | 40 | 0.028 | 0.025 | |
| | 37 | 0.833 | 0.550 | 0.533 |
| | 34 | 0.139 | 0.425 | 0.450 |
| | 32 | | | 0.017 |
| | *H* | 0.285 | 0.516 | 0.513 |
| Gp5-5 | 27 | 1.000 | 1.000 | 1.000 |
| Lap-2 | 83 | | 0.050 | |
| | 81 | 1.000 | 0.950 | 1.000 |
| | *H* | | 0.095 | |
| Lap-3 | 72 | | 0.050 | |
| | 70 | 1.000 | 0.950 | 0.966 |
| | 64 | | | 0.034 |
| | *H* | | 0.095 | 0.066 |
| Lap-4 | 54 | 1.000 | 1.000 | 1.000 |
| Lap-5 | 46 | 0.050 | 0.025 | 0.200 |
| | 44 | 0.575 | 0.150 | 0.167 |
| | 41 | 0.375 | 0.825 | 0.633 |
| | *H* | 0.526 | 0.296 | 0.531 |
| Ldh | 40 | | | 0.450 |
| | 36 | 1.000 | 1.000 | 0.550 |
| | *H* | | | 0.495 |
| Odh-1 | 61 | | | 0.100 |
| | 59 | 1.000 | 1.000 | 0.583 |
| | 56 | | | 0.317 |
| | *H* | | | 0.549 |
| Odh-2 | 44 | 0.525 | 1.000 | 1.000 |
| | 40 | 0.325 | | |
| | 38 | 0.150 | | |
| | *H* | 0.596 | | |

* Heterozygosity

locus Lap-4 was regarded as monomorphic, because it has one allele, *Lap-4*$^{54}$(Table 3).

In the Lap-2 locus, GX-L and Y-F had one allele of *Lap-2*$^{81}$, which was dominant in all populations; Y-L, however, had another allele (*Lap-2*$^{83}$). On the Lap-3 locus, GX-L showed monomorphism of the *Lap-3*$^{70}$ allele. This allele also was dominant in both Y-L and Y-F. Each population indicated heterozygosity with *Lap-3*$^{72}$ and *Lap-3*$^{70}$ alleles in Y-L, and *Lap-3*$^{70}$ and *Lap-3*$^{64}$ alleles in Y-F. In contrast, the Lap-5 locus showed polymorphism, constructed by 3 alleles in each population.

**Lactate dehydrogenase (Ldh):** Only Y-F indicated heterozygosity, constructed by the 2 alleles, *Ldh*$^{40}$ and *Ldh*$^{36}$ with nearly equal frequency (0.450 for *Ldh*$^{40}$ and 0.550 for *Ldh*$^{36}$). Both GX-L and Y-L populations showed monomorphism of allele *Ldh*$^{36}$.

**Octanol dehydrogenase (Odh):** At the locus Odh-1, GX-L and Y-L had one allele of *Odh-1*$^{59}$ (Table 3), while Y-F showed 5 genotypes constructed by 3 alleles, *Odh-1*$^{61}$, *Odh-1*$^{59}$ and *Odh-1*$^{56}$ (Table 2). At the locus Odh-2, in contrast, Y-L and Y-F populations had one allele of *Odh-2*$^{44}$. In GX-L, the *Odh-2*$^{44}$ allele was dominant in 3 alleles (*Odh-2*$^{44}$, *Odh-2*$^{40}$ and *Odh-2*$^{38}$).

### Genetic variabillity within a population

The genetic variability within a population, $P$ (polymorphic loci) and $\bar{H}$ (average heterozygosity per individual) is quantified in Table 4. The lowest

Table 4

Proportion of polymorphic loci ($P$) average heterozygosity ($\overline{H}$) calculated for 3 populations of *Anopheles minimus*.

| Populations | Proportion of polymorphic loci $P$ (20 loci) | Average heterozygosity $\overline{H}$ (20 loci) |
|---|---|---|
| GX-L | 0.500 | 0.220 |
| Y-L | 0.700 | 0.210 |
| Y-F | 0.700 | 0.340 |
| Average | 0.633 | 0.257 |

value of $P$ was obtained in GX-L (0.500) and the value was equal in Y-L and Y-F (0.700). The average $P$ value (0.633) among the 3 populations was a little higher than the usual level indicated by Nei (1975) who suggested that $P$ values of many kinds of organisms were usually found within 0.2-0.4. The highest value of $\overline{H}$ (0.340) was obtained from Y-F.

The 2 values of $P$ and $\overline{H}$ showed that Y-F was the most variable among the 3 populations.

**Genetic distance between populations**

The genetic diversity among the 3 populations in Nei's genetic distance ($D$) and identity ($I$) were calculated from the allele frequency of each population at the 20 respective protein loci (Table 5). The highest value of $D$ was 0.1946 between GX-L and Y-F. $D$ values of 0.1131 and 0.1069 were obtained between GX-L and Y-L, and between Y-L and Y-F, respectively. Based on the criteria by Nei (1975) and Thorpe (1982), these values were regarded as being within intraspecific variation. The dendrogram in Fig 2 indicates that GX-L is distant from the other 2 populations.

Table 5

Values of Nei's genetic identity ($I$) and genetic distance ($D$) among the 3 populations of *Anopheles minimus* based on 20 protein loci.

| $D$ | $I$ | | |
|---|---|---|---|
| | GX-L | Y-L | Y-F |
| GX-L | | 0.8931 | 0.8231 |
| Y-L | 0.1131 | | 0.8986 |
| Y-F | 0.1946 | 0.1069 | |

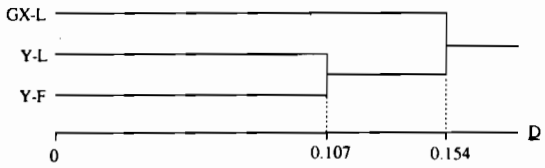


Fig 2–Dendrogram of the 3 populations of *Anopheles minimus* based on UPGMA clustering of the data of Table 5.

## DISCUSSION

Geographical variation of *An. minimus* in China has been discussed based on morphological and physiological studies (Ho, 1938; Liu *et al*, 1959). No study on genetic background using protein electrophoresis has previously been made. Yu and Li (1984) reported that there were 2 forms (A, B) of *An. minimus* on Hainan Island. These forms were classified by a detailed study of the morphological characteristics together with their susceptibility to DDT. Jiang *et al* (1986) found variation in the electrophoretic band patterns in non-specific esterase between forms A and B of this species in Yunnan Province. However, there has been no electrophoretic study made except on carboxylesterase. This, therefore, is the first attempt to classify the isozyme band pattern within the *An. minimus* species of China.

The level of genetic differentiation among the 3 populations of *An. minimus* obtained from Guangxi and Yunnan Provinces of China has been evaluated here on 20 protein loci. The dendrogram by UPGMA clustering based on the data of genetic distance ($D$) (Fig 2) clarifies that GX-L is far from the other 2 Yunnan populations, Y-L and Y-F. The result agrees with the morphological classification by Xu *et al* (unpublished) that the 2 Yunnan populations, Y-L and Y-F, are *An. minimus* form A and GX-L form B.

Nei (1975) described that the $D$ value between subspecies ranged within 0.004-0.351 (mainly 0.1-0.2). Thorpe (1982) concluded that between 2 populations, if the $D$ value was more than 0.16, they

could not be regarded as the same species, even if they were indentified as the same species by morphological and physiological characteristics and by the level of reproductive isolation. However, he then indicated that a $D$ range of 0.02-3.9 (mainly 0.22-1.6) appears to be an interspecific variation within a genus for a large number of organisms, except for birds. In the present results, Nei's genetic distance ($D$) was calculated as 0.1131 and 0.1964 between GX-L and Y-L and GX-L and Y-F, respectively (Table 5). Based on the above 2 criteria, the 3 *An. minimus* populations of China could be categorized as a single species. The differentiation between GX-L and Y- F is close to the level of a subspecies, however, more detailed data are needed to clarify their taxonomic status.

$P$ values quantifying the genetic variability within a population were calculated as 0.500 from GX-L and 0.700 from both Y-L and Y-F (Table 4). These values mean that 10 and 14 proteins of the 20 proteins examined show polymorphism. Each $P$ value is much larger than the usual, within a range of 0.2-0.4(average 0.3) (Nei, 1975). As shown in Table 4, $\overline{H}$ values (average 0.257) were also larger than the average $\overline{H}$, 0.1 (Nei, 1975). Kojima *et al* (1970) described that some enzymes, such as carboxylesterase, phosphatase and amylase, had high polymorphism in *Drosophila melanogaster* and *D. simulans*. The level of genetic variability in protein loci except glucose-metabolizing enzymes (*eg* carboxylesterase, alcohol dehydrogenase, octanol dehydrogenase and xanthine dehydrogenase) generally seems to be much higher than that of glucose-metabolizing enzymes (*eg* hexokinase, aldolase, isocitorate dehydrogenase, α-glycerophosphate, dehydrogenase glucose-6-phosphate dehydrogenase and phosphoglucomutase) (Lewontin, 1974). Of the 8 proteins examined in our study, all showed polymorphism in some populations. Our isozyme analysis might be carried out using the data from these higher polymorphic proteins. To estimate the genetic variability, further examinations using multiple numbers and kinds of protein are needed in the future.

Natural populations are usually used as much as possible in isozyme analysis. However, the GX-L and Y-L populations used in this study were larvae obtained from a laboratory colony. In particular, the Y-L population has been reared for about 2 years. The $P$ (0.700) and $\overline{H}$ (0.340) values of the field collected population Y-F, were higher than those of 2 laboratory populations. In *An. minimus* of Thailand, the highest values of $P$ (0.7) and $\overline{H}$ (0.36) were obtained from the population designated 'KCHT' (Tha Lam Yai in Kanchanaburi Province) among 8 field-collected populations based on 20 enzyme loci (Komalamisra, 1989). Laboratory colonies may lose a large amount of initial electrophoretic variation through inbreeding and inadvertent selection (Berlocher, 1984); the genetic differentiation between 2 of the populations could have undergone intensive acceleration due to random genetic drift (Mukai, 1977; Mukai *et al*, 1974, 1980) and the bottleneck effect (Chakraborty and Nei, 1977). Differentiation in the level of genetic variability within populations between Y-F, and GX-L and Y-L, may have been due to this finding.

In the present study we carried out a fundamental investigation mixing the data obtained from a field-collected population and 2 laboratory maintained populations. However, the results obtained are still valuable for estimating the genetic variability within populations and the genetic differentiation between them.

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