

## Gene Constitution of Egg White Proteins of Native Chicken in Asian Countries

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**ABSTRACT :** Genetic variations of seven egg white protein loci in 1,112 samples from eight Asian countries (Yunnan province of China, Mongolia, Nepal, Vietnam, Laos, Thailand, Myanmar, Indonesia) and 360 samples from two improved breeds (Isa Brown, Boris Brown) were investigated by using starch gel and polyacrylamide gel electrophoresis. Five egg white protein loci (*Ov*, *G<sub>3</sub>*, *G<sub>2</sub>*, *G<sub>1</sub>* and *Tf<sub>EW</sub>*) were found to be polymorphic in Asian native chicken populations. The proportion of polymorphic loci ( $P_{pol}$ ) and average heterozygosity ( $\bar{H}$ ) of Asian native populations varied from 0.143 to 0.714 and 0.014 to 0.225, respectively, and were higher than those of improved breeds. The subdivision index ( $G_{ST}$ ) value among 18 native chicken populations in Asia is lower (0.0827) than among improved chicken populations (0.1693). This value means that the degree of subdivision among Asian native populations is lower than among improved breeds and gene constitutions among populations in Asia are similar. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 2 : 157-165)

**Key Words :** Native Chicken, Egg White, Protein Polymorphism, Genetic Variability, Heterozygosity

### INTRODUCTION

Egg is valuable balanced food containing essential amino acid for human, and is utilized, not only for eating, but also as materials of processed food, preservatives, additives of medicine, cosmetic material, etc.. The egg white protects the yolk from physical impact and contains essential nutrients for embryo development, as well as physiologically active and antibacterial materials. In rural area of Asian countries, people have kept the native chickens, which is important animal protein source and has significant value for light cashing.

Polymorphisms of egg white proteins have been reported, mainly in improved breeds, in flavoprotein (*Rd*), ovalbumin (*Ov*), ovoglobulin (*G<sub>3</sub>* and *G<sub>2</sub>*), ovotransferrin (*Tf<sub>EW</sub>*), ovomacroglobulin (*Omg*) and lysozyme (*G<sub>1</sub>*) (Baker, 1964, 1968; Lush, 1961; Buvanendran, 1967; William, 1967). It is very important to assess the genetic variability on egg white protein of native chicken populations in Asia. Because analysis of egg white protein polymorphism in Asian native chicken populations may lead to the detection of new phenotypes and alleles, and may be useful to assess the degree of genetic variability of native chicken populations in Asia and differences in egg white protein loci between improved breed and native chicken. Sampling of

Egg White for electrophoresis from native chicken is difficult compare to the sampling from improved breeds, because of low egg production of native chicken and embryonic development by their brooding. A few studies (Maeda et al., 1992; Inafuku et al., 1998) have been reported on egg white protein polymorphism of native chicken populations in Asia. Rare variants were found in ovalbumin (*A<sub>1</sub>* defective; Inafuku et al., 1997), and lysozyme (slow band; Inafuku et al., 1998) in native chickens in Japan and Indonesia, respectively. We have conducted, since 1993, the collections of blood and egg white samples from native chicken in Nepal (Maeda et al., 1992), China (Maeda et al., 1995), Indonesia (Inafuku et al., 1998), Mongolia (Maeda, et al., 1999), Vietnam, Laos, Thailand and Myanmar. In this study, we analyzed gene constitution of egg white protein of native chicken of eight Asian countries, and compared those with some improved breeds.

### MATERIALS AND METHODS

#### Egg white samples

During 1992 to 1999, total 1112 egg white samples were collected from native chicken populations in 8 Asian countries (Yunnan Province in China, Mongolia, Nepal, Vietnam, Laos, Thailand, Myanmar, Indonesia) (figure 1). To avoid double sampling, each egg sample was collected from a different farmer (table 1). As mentioned above, not all collected eggs were available for electrophoresis analysis, because embryonic development was found in about half of collected eggs. Each egg was opened on glassware and thick albumen of fresh egg was collected by syringe. Albumen was kept in plastic tube with dry ice and was transported to laboratory of Animal Genetics and

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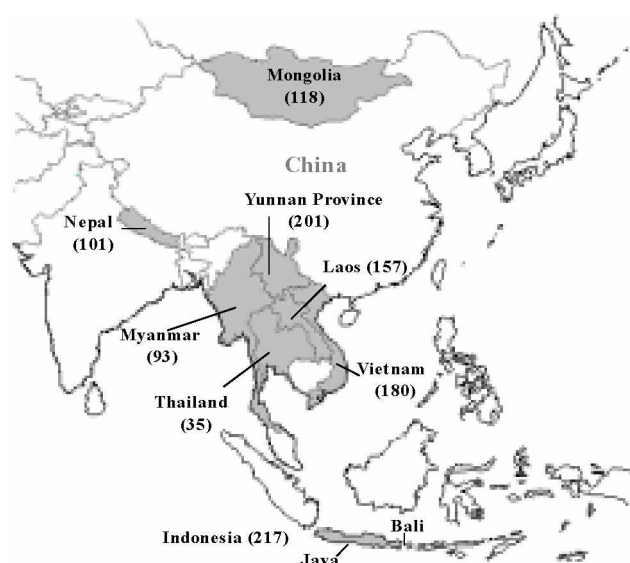
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**Table 1.** Country and location of egg sample collection of Asian native chicken

| Country             | Area / (Total Number) | Sampling location | No. of samples  | Reference             |     |  |
|---------------------|-----------------------|-------------------|-----------------|-----------------------|-----|--|
| Vietnam             | North (98)            | Lan Son           | 15              |                       |     |  |
|                     |                       | Thai Nguyen       | 20              |                       |     |  |
|                     |                       | Quang Ninh        | 15              |                       |     |  |
|                     |                       | Ha Tay            | 20              |                       |     |  |
|                     |                       | Thanh Hoa         | 15              |                       |     |  |
|                     |                       | Nghe An           | 13              |                       |     |  |
|                     | South (82)            | Phan Rang         | 17              |                       |     |  |
|                     |                       | Phan Tiet         | 20              |                       |     |  |
|                     |                       | Ba Ria            | 5               |                       |     |  |
|                     |                       | Chau Doc          | 10              |                       |     |  |
|                     |                       | Can Tho           | 15              |                       |     |  |
|                     |                       | Soc Trang         | 15              |                       |     |  |
|                     |                       | Laos              | North (60)      | Luang Namtha          | 60  |  |
| Central (67)        | Luang Prabang         |                   | 15              |                       |     |  |
|                     | Xiengkhuang           |                   | 16              |                       |     |  |
|                     | Vientiane             |                   | 7               |                       |     |  |
|                     | Borikhamxay           |                   | 17              |                       |     |  |
| South (30)          | Xam Nua               |                   | 12              |                       |     |  |
|                     | Champasack            |                   | 13              |                       |     |  |
|                     | Attapu                |                   | 17              |                       |     |  |
| Thailand            | South (35)            |                   | Prachuapkirikan | 35                    |     |  |
| Myanmar             | Yangon (53)           |                   | Okkan           | 3                     |     |  |
|                     |                       | Kayan             | 12              |                       |     |  |
|                     |                       | Pindaya           | 5               |                       |     |  |
|                     |                       | Yanngshwe         | 8               |                       |     |  |
|                     |                       | Yangon            | 16              |                       |     |  |
|                     |                       | Bago              | 9               |                       |     |  |
|                     |                       | Nepal             | Mandalay (40)   | Mandalay              | 40  |  |
|                     |                       |                   | West (64)       | Jomson road           | 24  |  |
| Pokhara             | 40                    |                   |                 |                       |     |  |
| East (37)           | Solu Khumbu           |                   | 18              | Maeda et al. (1992)   |     |  |
| China <sup>1)</sup> | Chahua chicken        | Yunnan province   | 79              |                       |     |  |
|                     | Xishuangbanna game    | Xishuangbanna     | 10              |                       |     |  |
|                     | Xishuangbanna native  | Xishuangbanna     | 29              | Maeda et al. (1995)   |     |  |
|                     | Wuding chicken        | Wuding            | 45              |                       |     |  |
|                     | Yangbi Huang chicken  | Yangbi            | 38              |                       |     |  |
| Indonesia           | Bali (106)            | Pedungan          | 11              |                       |     |  |
|                     |                       | Kesiman           | 25              |                       |     |  |
|                     |                       | Kamasan           | 20              |                       |     |  |
|                     |                       | Karangasem        | 23              |                       |     |  |
|                     |                       | Klungkung         | 27              |                       |     |  |
|                     |                       | Java (111)        | Karawang        | 15                    |     |  |
|                     |                       | Jakarta           | 12              | Inafuku et al. (1998) |     |  |
|                     |                       | Jember            | 18              |                       |     |  |
|                     |                       | Semarang          | 6               |                       |     |  |
|                     |                       | Surakarta         | 23              |                       |     |  |
|                     |                       | Purwodadi         | 20              |                       |     |  |
|                     |                       | Ungaran           | 17              |                       |     |  |
|                     |                       | Mongolia          | - (128)         | Sukhbaatar            | 110 |  |
| Ulaanbaatar         | 18                    |                   |                 | Maeda et al. (1999)   |     |  |
| Total               | 1112                  |                   |                 |                       |     |  |

1) breed in Yunnan Province of China.



**Figure 1.** Surveyed countries and number of egg white examined in Asia

( ) : number of egg white sample

Breeding. Kagoshima University, Japan to analyze egg white protein polymorphism.

For comparison between native chicken and improved breeds, a total of 360 egg white samples of Isa Brown and Boris Brown were collected at Poultry Experimental Station of Kagoshima Prefecture and were analyzed for egg white protein polymorphism.

### Electrophoresis

Electrophoretic separation of Ovalbumin ( $Ov$ ), Ovoglobulin ( $G_2$ ), Ovotransferrin ( $Tf_{EW}$ ), Ovomacroglobulin ( $Omg$ ), Flavoprotein ( $Rd$ ) was performed in 8-12% native polyacrylamide gel (Laemmli, 1970). For ovoglobulin ( $G_3$ ) and  $G_1$  electrophoresis was performed in 10-12% starch gel electrophoresis by the methods of Stratil (1968) or Baker and Manwell (1962). All electrophoresis were carried out at 4°C. Gels were stained with 5% amide black 10B solution.

### Calculation of genetic variability

Genetic variabilities were calculated as proportion of polymorphic loci ( $P_{poly}$ ) and average heterozygosity ( $\bar{H}$ ).  $P_{poly}$  was calculated as the ratio of polymorphic loci to the total loci analyzed. Average heterozygosity ( $\bar{H}$ ) was estimated the Nei's formular (1978).  $\bar{H} = 2n(1 - \sum q_i^2) / (2n-1)$ , where  $q_i$  is the frequency of  $i^{th}$  allele at a locus,  $n$  is the number of individuals in the population, and average means the average over all loci examined.

Relative magnitude of gene differentiation among sub populations was estimated using the coefficient of gene differentiation,  $G_{ST}$  (Nei, 1973). The coefficient is given by  $G_{ST} = (\bar{H}_T - \bar{H}_S) / \bar{H}_T$ , where  $\bar{H}_T$  and  $\bar{H}_S$  are the average

heterozygosity of the total populations calculated from average gene frequencies of sub populations and the mean average heterozygosity of the local population, respectively. Standard Genetic distance ( $D_S$ ) was calculated by the formula given by Nei (1972).  $D_S = -\log_e I$ ,  $I = J_{xy} / (J_x J_y)^{1/2}$  where  $J_x$ ,  $J_y$  and  $J_{xy}$  are the averages of  $\sum x_i^2$ ,  $\sum y_i^2$  and  $\sum x_i y_i$  over loci studied, respectively.  $x_i$  and  $y_i$  are the frequencies of the  $i^{th}$  allele at a locus in X and Y populations, respectively. The phylogenetic tree constructed using the neighbor-joining method (Saitou and Nei, 1987) by using the MEGA software (Kumar, 1993). To compare the genetic variability among improved breeds and Asian native populations published data of White Leghorn and White Cornish was used.

## RESULTS AND DISCUSSION

### Gene frequency and genetic variability

Table 2 shows the distribution of gene frequencies of egg white protein (seven loci) of 27 populations of Asian native chicken and improved breed. Out of 7 loci,  $Ov$ ,  $G_3$ ,  $G_2$ ,  $G_1$  and  $Tf_{EW}$  were polymorphic (figure 2). The  $Omg$  and  $Rd$  loci were monomorphic in all areas and breeds. Polymorphism at the  $G_1$  locus was found in the Java population from Indonesia only.

In the comparison between Asian native populations and improved breeds, the  $G_3^A$  allele was not found in all populations of improved breeds, but was distributed in some Asian native populations. Although almost all Asian native populations showed polymorphism in the  $Tf_{EW}$  locus except two populations from China (Wuding chicken, Yangbi huang chicken), improved breeds except one of the White Cornish were fixed in the  $Tf_{EW}^B$  allele. The gene frequency of the  $Ov^A$  is higher (0.848-1.000) than the  $Ov^B$  in almost all native populations in Asia except the Thailand-south population.

In the breeds from the Yunnan province of China, the  $Ov^A$  allele was fixed in the Wuding chicken, the Xishuangbanna game chicken, the Xishuangbanna native chicken and the Yangbi huang chicken, and the  $Tf_{EW}^A$  is not present in all breeds. For fixation of the  $Ov^A$  allele and disappearance of the  $Tf_{EW}^A$  allele in four breeds, possible reasons include: The  $Ov^B$  and the  $Tf_{EW}^A$  disappeared because of genetic drift during keeping in a small colony, these alleles had been eliminated by a bottle neck effect. We have no information how many birds were present at the initial generation in those closed flocks.

Native chickens in rural area of other Asian countries are mated randomly and transported, sometimes, to other villages as gifts. Random mating in back yard and movement among villages (migration) may be result in gene flow among villages and maintain minor genes at low frequency.  $G_2^L$  in Nepal-West and  $G_1^S$  in Indonesia-Java are

**Table 2.** Distribution of gene frequencies of egg white protein loci in 27 populations including native chicken and improved breeds

| Code                 | Population                 | N    | $O_V$   |         | $G_3$   |         |         | $G_2$   |         |         | $Tf_{EW}$   |             |             | $G_1$   |         |
|----------------------|----------------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|-------------|-------------|-------------|---------|---------|
|                      |                            |      | $O_V^A$ | $O_V^B$ | $G_3^A$ | $G_3^B$ | $G_3^J$ | $G_2^A$ | $G_2^B$ | $G_2^L$ | $Tf_{EW}^A$ | $Tf_{EW}^B$ | $Tf_{EW}^C$ | $G_1^F$ | $G_1^S$ |
| Asian native chicken |                            |      |         |         |         |         |         |         |         |         |             |             |             |         |         |
| 1.                   | Vietnam-North              | 98   | 0.883   | 0.117   | 0.724   | 0.148   | 0.128   | 0.092   | 0.908   | 0       | 0.041       | 0.730       | 0.230       | 1       | 0       |
| 2.                   | Vietnam-South              | 82   | 0.848   | 0.152   | 0.720   | 0.226   | 0.054   | 0.402   | 0.598   | 0       | 0.140       | 0.750       | 0.110       | 1       | 0       |
| 3.                   | Laos-North                 | 60   | 0.917   | 0.083   | 0.575   | 0.283   | 0.142   | 0.175   | 0.825   | 0       | 0.025       | 0.933       | 0.042       | 1       | 0       |
| 4.                   | Laos-Central               | 67   | 0.918   | 0.082   | 0.597   | 0.119   | 0.284   | 0.172   | 0.828   | 0       | 0.045       | 0.866       | 0.090       | 1       | 0       |
| 5.                   | Laos-South                 | 30   | 0.900   | 0.100   | 0.633   | 0.233   | 0.134   | 0.050   | 0.950   | 0       | 0           | 0.833       | 0.167       | 1       | 0       |
| 6.                   | China-Chahua chicken       | 79   | 0.899   | 0.101   | 0.949   | 0.007   | 0.044   | 0.171   | 0.829   | 0       | 0           | 0.861       | 0.139       | 1       | 0       |
| 7.                   | China-Xishuangbanna game   | 10   | 1       | 0       | 1       | 0       | 0       | 0       | 1       | 0       | 0           | 0.950       | 0.050       | 1       | 0       |
| 8.                   | China-Xishuangbanna native | 29   | 1       | 0       | 0.724   | 0.241   | 0.035   | 0.155   | 0.845   | 0       | 0           | 0.983       | 0.017       | 1       | 0       |
| 9.                   | China-Wuding chicken       | 45   | 1       | 0       | 0.822   | 0.178   | 0       | 0.122   | 0.878   | 0       | 0           | 1           | 0           | 1       | 0       |
| 10.                  | China-Yangbi huang chicken | 38   | 1       | 0       | 0.750   | 0.250   | 0       | 0.105   | 0.895   | 0       | 0           | 1           | 0           | 1       | 0       |
| 11.                  | Indonesia-Java island      | 111  | 0.996   | 0.004   | 0.784   | 0.203   | 0.013   | 0.056   | 0.944   | 0       | 0           | 0.983       | 0.017       | 0.979   | 0.021   |
| 12.                  | Indonesia-Bali island      | 106  | 1       | 0       | 0.779   | 0.221   | 0       | 0.085   | 0.915   | 0       | 0           | 0.986       | 0.014       | 1       | 0       |
| 13.                  | Nepal-West                 | 64   | 0.934   | 0.066   | 0.669   | 0.311   | 0.020   | 0.086   | 0.904   | 0.010   | 0.010       | 0.865       | 0.125       | 1       | 0       |
| 14.                  | Nepal-East                 | 37   | 0.916   | 0.084   | 0.833   | 0.167   | 0       | 0       | 1       | 0       | 0.028       | 0.875       | 0.097       | 1       | 0       |
| 15.                  | Mongolia                   | 118  | 1       | 0       | 0.648   | 0.352   | 0       | 0.199   | 0.801   | 0       | 0.004       | 0.987       | 0.009       | 1       | 0       |
| 16.                  | Myanmar-Yangon             | 48   | 0.937   | 0.063   | 0.719   | 0.156   | 0.125   | 0.240   | 0.760   | 0       | 0.188       | 0.792       | 0.020       | 1       | 0       |
| 17.                  | Myanmar-Mandalay           | 45   | 0.900   | 0.100   | 0.811   | 0.078   | 0.111   | 0.189   | 0.811   | 0       | 0.111       | 0.833       | 0.056       | 1       | 0       |
| 18.                  | Thailand-South             | 35   | 0.543   | 0.457   | 0.671   | 0.157   | 0.172   | 0.257   | 0.743   | 0       | 0.029       | 0.971       | 0           | 1       | 0       |
| Improved breed       |                            |      |         |         |         |         |         |         |         |         |             |             |             |         |         |
| 19.                  | Boris Brown                | 276  | 0.933   | 0.067   | 0.524   | 0.476   | 0       | 0.417   | 0.583   | 0       | 0           | 1           | 0           | 1       | 0       |
| 20.                  | Isa Brown                  | 84   | 1       | 0       | 0.482   | 0.518   | 0       | 0.458   | 0.542   | 0       | 0           | 1           | 0           | 1       | 0       |
| 21.                  | White Leghorn (WL-C36)     | 289  | 0.955   | 0.045   | 0.995   | 0.005   | 0       | 0.388   | 0.612   | 0       | 0           | 1           | 0           | 1       | 0       |
| 22.                  | White Leghorn (WL-C37)     | 1218 | 0.655   | 0.345   | 0.758   | 0.242   | 0       | 0.090   | 0.910   | 0       | 0           | 1           | 0           | 1       | 0       |
| 23.                  | White Leghorn (WL-S2)      | 949  | 0.550   | 0.450   | 0.851   | 0.149   | 0       | 0       | 1       | 0       | 0           | 1           | 0           | 1       | 0       |
| 24.                  | White Leghorn (WL-S5)      | 873  | 1       | 0       | 0.580   | 0.420   | 0       | 0       | 1       | 0       | 0           | 1           | 0           | 1       | 0       |
| 25.                  | White Cornish (WC-1)       | 370  | 0.986   | 0.014   | 0.441   | 0.559   | 0       | 0.227   | 0.773   | 0       | 0           | 0.984       | 0.016       | 1       | 0       |
| 26.                  | White Cornish (WC-2)       | 100  | 0.985   | 0.015   | 0.490   | 0.510   | 0       | 0.265   | 0.735   | 0       | 0           | 1           | 0           | 1       | 0       |
| 27.                  | White Cornish (WC-3)       | 45   | 1       | 0       | 0.520   | 0.480   | 0       | 0.230   | 0.770   | 0       | 0           | 1           | 0           | 1       | 0       |

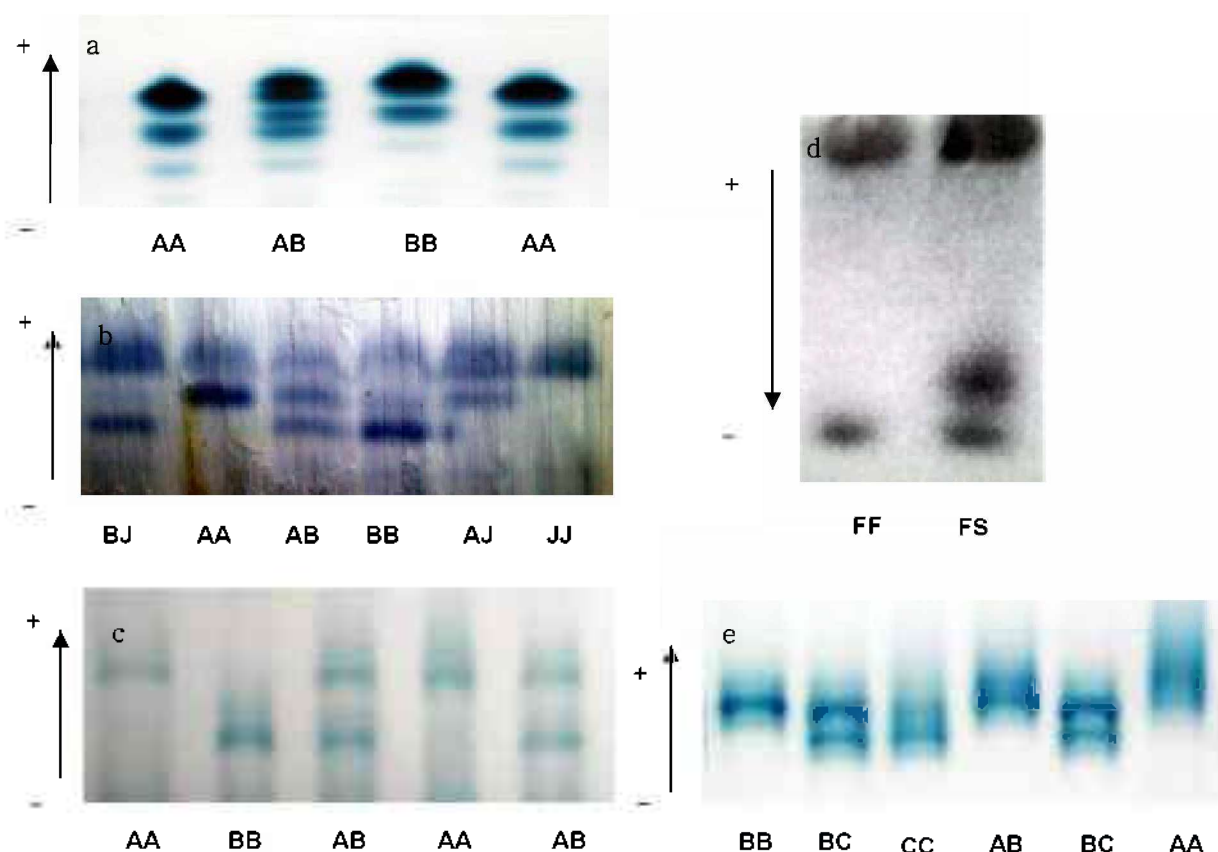
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rare alleles but are found also in Europe (Baker, 1968, 1970).

The genetic variability of 27 populations of Asian native chicken and improved breeds is given in table 3. The range of heterozygosity is 0.014-0.225 in Asian native populations and 0.070-0.159 in improved breeds. The values for native chicken tend to higher than for improved breeds. But there is no significant difference between native chicken and improved chicken, the range of heterozygosities of native chicken in four countries (Vietnam, Laos, Thailand and Myanmar) in the Indochina peninsula are relatively high (0.154-0.225). Native chicken groups in Mongolia, the Yunnan province in China, Indonesia and Nepal-east showed relatively low

heterozygosity (0.014-0.094). The Xishuangbanna game population is the lowest in both  $P_{poly}$  (0.143) and average heterozygosity (0.014) since it has been maintained in small flocks. Heterozygosities of Java and Bali populations were relatively low (0.076).

Some studies reported the heterozygosity of Asian native chicken based on blood protein polymorphism (Hashiguchi et al., 1993; Maeda et al., 1992; Okamoto et al., 1999). The heterozygosities of native chicken in Indonesia, Nepal and Laos were calculated as 0.0757, 0.1176 and 0.1640, respectively. These values among the three countries are parallel to  $\bar{H}$  values from egg white protein polymorphisms. Although the heterozygosity of Indonesia-Java was low,  $P_{poly}$  was the highest because of



**Figure 2.** Electrophoretic phenotypes of polymorphic egg white protein loci observed in Asian native chicken populations (a) Ovalbumin. (b) Ovoglobulin  $G_3$ , (c) Ovoglobulin  $G_2$ , (d) Lysozyme. (e) Ovotransferrin

polymorphism in 5 loci except the *Omg* locus.

Heterozygosities of improved breeds (0.070-0.159) were lower than those of birds from 4 countries of the Indochina peninsula. The Boris and Isa Brown varieties were higher than the White Leghorn and White Cornish.

#### Degree of subdivision among populations

Table 4 shows the subdivision index ( $G_{ST}$ ) from pooled data and in various comparisons. The subdivision index ( $G_{ST}$ ) was calculated in 0.1287 among 27 populations. The value among 18 native chicken populations of Asia (0.0827) is lower than it among improved chicken population (0.1693). These values mean that the degree of subdivision among native chicken populations in Asia is lower than among improved breeds and gene constitutions among Asian native chicken populations are similar in each other compare to improved breeds. The  $G_{ST}$  values among local populations in each country of Asia were calculated in 0.0011-0.0399 meaning small degree of subdivision. The large value of  $G_{ST}$  of improved breeds, compare to among Asian native populations, means that differentiation among improved breeds, in the gene constitution of the egg white protein, is larger than the degree of subdivision among native chicken populations.

In improved breeds, although the values of  $G_{ST}$  in Browns and White Cornish are small (0.0027-0.0031), but large in White Leghorn (0.1961). We could not have the information of breeding history of White Leghorn cited here. The White Leghorn has been improved for high egg production in various countries. During the process of improvement in various countries, gene constitution of egg white protein might be changed by genetic drift and, as result, degree of subdivision among lines became large.

#### Genetic distance

The genetic distances within and among chicken groups are presented in table 5. The genetic distances among 18 populations of Asian native chicken and 16 populations excluded Vietnam-South and Thailand-South was 0.000-0.063 and 0.000-0.029, respectively. In the comparison among improved breeds, the genetic distance among four lines of White Leghorn were calculated as 0.005-0.065 and was higher than the values among Boris and Isa Browns 0.002 and among lines of White Cornish 0.000-0.001. The genetic distance between White Leghorn and Browns or White Cornish were relatively high (0.034-0.113 and 0.010-0.082, respectively). These results suggest that the genetic constitution of White Leghorn is differs among these lines compare to those of White Cornish and Browns.

**Table 3.** Proportion of polymorphic loci ( $P_{poly}$ ), average heterozygosity ( $\bar{H}$ ) in egg white protein of 27 populations of Asian native chicken and improved breeds

| Population / Breed         | No. of samples | $P_{poly} \pm S.E.$ | $\bar{H} \pm S.E.$ |
|----------------------------|----------------|---------------------|--------------------|
| Asian native chicken       |                |                     |                    |
| Vietnam-North              | 98             | 0.571±0.202         | 0.175±0.072        |
| Vietnam-South              | 82             | 0.571±0.202         | 0.225±0.083        |
| Laos-North                 | 60             | 0.571±0.202         | 0.162±0.079        |
| Laos-Central               | 67             | 0.571±0.202         | 0.175±0.077        |
| Laos-South                 | 30             | 0.571±0.202         | 0.154±0.074        |
| Thailand-South             | 35             | 0.571±0.202         | 0.204±0.091        |
| Myanmar-Yangon             | 53             | 0.571±0.202         | 0.180±0.074        |
| Myanmar-Mandalay           | 40             | 0.571±0.202         | 0.157±0.058        |
| Nepal-West                 | 64             | 0.571±0.202         | 0.141±0.063        |
| Nepal-East                 | 37             | 0.429±0.202         | 0.094±0.046        |
| China-Chahua chicken       | 79             | 0.571±0.202         | 0.115±0.046        |
| China-Xishuangbanna game   | 10             | 0.143±0.143         | 0.014±0.014        |
| China-Xishuangbanna native | 29             | 0.429±0.202         | 0.102±0.064        |
| China-Wuding chicken       | 45             | 0.286±0.184         | 0.072±0.048        |
| China-Yangbi Huang chicken | 38             | 0.286±0.184         | 0.080±0.056        |
| Mongolia                   | 128            | 0.429±0.202         | 0.114±0.072        |
| Indonesia-Java island      | 111            | 0.714±0.184         | 0.076±0.047        |
| Indonesia-Bali island      | 106            | 0.429±0.202         | 0.075±0.050        |
| range                      |                | 0.143–0.714         | 0.014–0.225        |
| Improved breed             |                |                     |                    |
| Boris Brown                | 276            | 0.429±0.202         | 0.159±0.060        |
| Isa Brown                  | 84             | 0.286±0.184         | 0.142±0.060        |
| White Leghorn(C36)         | 289            | 0.429±0.202         | 0.082±0.059        |
| White Leghorn(C37)         | 1218           | 0.429±0.202         | 0.140±0.059        |
| White Leghorn(S2)          | 949            | 0.286±0.184         | 0.107±0.059        |
| White Leghorn(S5)          | 873            | 0.143±0.143         | 0.070±0.059        |
| White Cornish(1)           | 370            | 0.571±0.202         | 0.129±0.058        |
| White Cornish(2)           | 100            | 0.429±0.202         | 0.131±0.058        |
| White Cornish(3)           | 45             | 0.286±0.184         | 0.122±0.058        |
| range                      |                | 0.143–0.429         | 0.070–0.159        |

**Table 4.** Estimation of subdivision index ( $G_{ST}$ ) in various countries

|   |        | $H_s$  | $G_{ST}$ |
|---|--------|--------|----------|
| Among 27 populations                              | 0.1445 | 0.1259 | 0.1287   |
| Among countries within native chicken             | 0.1403 | 0.1287 | 0.0827   |
| Among sub population within Vietnam populations   | 0.2080 | 0.1997 | 0.0399   |
| Among sub population within Laos populations      | 0.1671 | 0.1640 | 0.0186   |
| Among sub population within China populations     | 0.0823 | 0.0765 | 0.0058   |
| Among sub population within Indonesia populations | 0.0758 | 0.0757 | 0.0035   |
| Among sub population within Nepal populations     | 0.1200 | 0.1176 | 0.0200   |
| Among sub population within Myanmar populations   | 0.1700 | 0.1689 | 0.0011   |
| Among line within improved chicken                | 0.1447 | 0.1202 | 0.1693   |
| Among sub population within Brown                 | 0.1509 | 0.1505 | 0.0027   |
| Among sub population within White Leghorn         | 0.1239 | 0.0996 | 0.1961   |
| Among sub population within White Cornish         | 0.1278 | 0.1274 | 0.0031   |

**Table 5.** Standard genetic distances (Ds) matrix obtained from the frequencies of seven egg white proteins of 27 populations including native chicken and improved breeds

| population                     | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    | 21    | 22    | 23    | 24    | 25    | 26    | 27   |
|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|
| 1. Vietnam-North               | ----  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 2. Vietnam-South               | 0.025 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 3. Laos-North                  | 0.014 | 0.020 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 4. Laos-Central                | 0.010 | 0.023 | 0.006 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 5. Laos-South                  | 0.004 | 0.031 | 0.007 | 0.008 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 6. China-Chahua chicken        | 0.011 | 0.023 | 0.024 | 0.019 | 0.019 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 7. China-Xishuangbanna game    | 0.017 | 0.046 | 0.029 | 0.027 | 0.020 | 0.007 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 8. China-Xishuangbanna native  | 0.015 | 0.022 | 0.004 | 0.012 | 0.010 | 0.015 | 0.015 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 9. China-Wuding chicken        | 0.016 | 0.026 | 0.010 | 0.015 | 0.012 | 0.010 | 0.008 | 0.001 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 10. China-Yangbi Huang chicken | 0.016 | 0.028 | 0.007 | 0.015 | 0.010 | 0.016 | 0.013 | 0.001 | 0.001 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 11. Indonesia-Java             | 0.013 | 0.033 | 0.009 | 0.015 | 0.009 | 0.013 | 0.008 | 0.002 | 0.001 | 0.001 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 12. Indonesia-Bali             | 0.014 | 0.029 | 0.008 | 0.015 | 0.009 | 0.013 | 0.009 | 0.001 | 0.001 | 0.000 | 0.000 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 13. Nepal-West                 | 0.008 | 0.025 | 0.005 | 0.013 | 0.003 | 0.018 | 0.019 | 0.005 | 0.007 | 0.004 | 0.005 | 0.004 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 14. Nepal-East                 | 0.007 | 0.035 | 0.016 | 0.018 | 0.007 | 0.010 | 0.006 | 0.009 | 0.006 | 0.007 | 0.004 | 0.005 | 0.006 | ****  |       |       |       |       |       |       |       |       |       |       |       |       |      |
| 15. Mongolia                   | 0.023 | 0.022 | 0.004 | 0.017 | 0.014 | 0.026 | 0.028 | 0.002 | 0.006 | 0.003 | 0.008 | 0.005 | 0.006 | 0.017 | ****  |       |       |       |       |       |       |       |       |       |       |       |      |
| 16. Myanmar-Yangon             | 0.013 | 0.009 | 0.010 | 0.009 | 0.015 | 0.014 | 0.023 | 0.010 | 0.011 | 0.012 | 0.014 | 0.012 | 0.013 | 0.017 | 0.013 | ****  |       |       |       |       |       |       |       |       |       |       |      |
| 17. Myanmar-Mandalay           | 0.008 | 0.014 | 0.012 | 0.009 | 0.012 | 0.004 | 0.012 | 0.009 | 0.008 | 0.011 | 0.010 | 0.009 | 0.012 | 0.009 | 0.017 | 0.003 | ****  |       |       |       |       |       |       |       |       |       |      |
| 18. Thailand-South             | 0.044 | 0.036 | 0.034 | 0.036 | 0.042 | 0.042 | 0.063 | 0.046 | 0.048 | 0.049 | 0.051 | 0.050 | 0.045 | 0.047 | 0.050 | 0.041 | 0.034 | ****  |       |       |       |       |       |       |       |       |      |
| 19. Boris Brown                | 0.054 | 0.022 | 0.019 | 0.038 | 0.043 | 0.057 | 0.074 | 0.023 | 0.034 | 0.029 | 0.039 | 0.034 | 0.030 | 0.056 | 0.013 | 0.031 | 0.042 | 0.055 | ****  |       |       |       |       |       |       |       |      |
| 20. Isa Brown                  | 0.067 | 0.030 | 0.027 | 0.048 | 0.055 | 0.071 | 0.089 | 0.031 | 0.043 | 0.038 | 0.050 | 0.043 | 0.040 | 0.070 | 0.019 | 0.040 | 0.054 | 0.074 | 0.002 | ****  |       |       |       |       |       |       |      |
| 21. White Leghorn (WL-C36)     | 0.040 | 0.020 | 0.036 | 0.036 | 0.047 | 0.013 | 0.027 | 0.023 | 0.019 | 0.027 | 0.029 | 0.026 | 0.039 | 0.036 | 0.030 | 0.021 | 0.016 | 0.051 | 0.043 | 0.052 | ****  |       |       |       |       |       |      |
| 22. White Leghorn (WL-C37)     | 0.026 | 0.038 | 0.022 | 0.031 | 0.022 | 0.026 | 0.032 | 0.024 | 0.023 | 0.022 | 0.022 | 0.022 | 0.020 | 0.018 | 0.028 | 0.032 | 0.023 | 0.012 | 0.049 | 0.068 | 0.045 | ****  |       |       |       |       |      |
| 23. White Leghorn (WL-S2)      | 0.039 | 0.063 | 0.045 | 0.050 | 0.037 | 0.036 | 0.040 | 0.047 | 0.041 | 0.043 | 0.039 | 0.041 | 0.040 | 0.028 | 0.056 | 0.053 | 0.037 | 0.018 | 0.087 | 0.113 | 0.065 | 0.005 | ****  |       |       |       |      |
| 24. White Leghorn (WL-S5)      | 0.025 | 0.048 | 0.010 | 0.024 | 0.012 | 0.040 | 0.031 | 0.009 | 0.013 | 0.007 | 0.009 | 0.009 | 0.006 | 0.016 | 0.008 | 0.028 | 0.029 | 0.063 | 0.034 | 0.041 | 0.061 | 0.030 | 0.052 | ****  |       |       |      |
| 25. White Cornish (WC-1)       | 0.045 | 0.038 | 0.013 | 0.034 | 0.029 | 0.062 | 0.060 | 0.019 | 0.029 | 0.021 | 0.029 | 0.025 | 0.018 | 0.043 | 0.009 | 0.034 | 0.044 | 0.067 | 0.009 | 0.011 | 0.065 | 0.046 | 0.082 | 0.013 | ****  |       |      |
| 26. White Cornish (WC-2)       | 0.042 | 0.030 | 0.011 | 0.030 | 0.028 | 0.054 | 0.060 | 0.015 | 0.024 | 0.017 | 0.025 | 0.022 | 0.017 | 0.040 | 0.006 | 0.028 | 0.038 | 0.061 | 0.005 | 0.007 | 0.052 | 0.043 | 0.078 | 0.014 | 0.001 | ****  |      |
| 27. White Cornish (WC-3)       | 0.036 | 0.030 | 0.009 | 0.026 | 0.023 | 0.047 | 0.051 | 0.011 | 0.019 | 0.013 | 0.019 | 0.016 | 0.013 | 0.033 | 0.003 | 0.025 | 0.033 | 0.060 | 0.008 | 0.011 | 0.049 | 0.039 | 0.073 | 0.010 | 0.001 | 0.000 | **** |

### Dendrogram

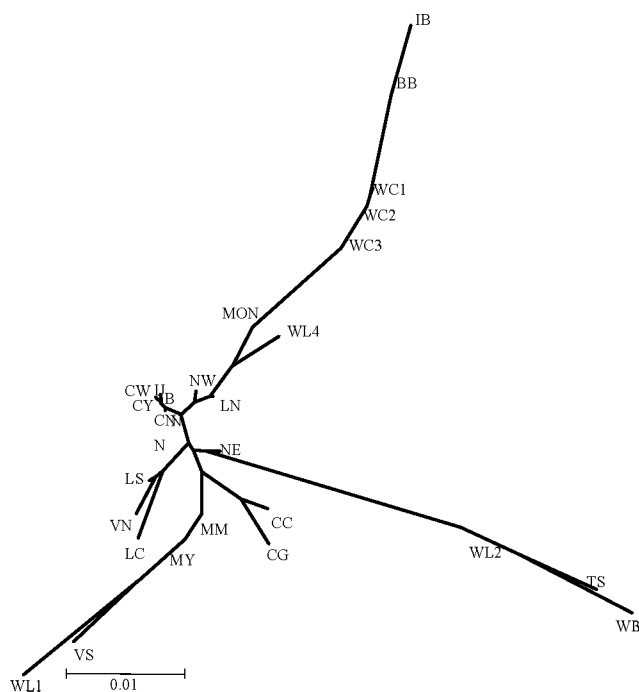
Figure 3 shows the phylogenetic tree of 27 populations including native chicken and improved breeds drawn by using neighbor-joining method (Saitou and Nei, 1987) by MEGA software (Kumar, 1993).

The 27 populations roughly formed themselves in three branches; the first one is composed of Browns (IB and BB) and White Cornish (WC1, WC2 and WC3), the second one is two White Leghorns (WL2, WL3) and native chicken in Thailand, and the third one is a White Leghorn (WL1) and native chicken in Vietnam-South. Almost Asian native chicken populations except Thailand-South and Vietnam-South are distributed in the center of three branches. The phase of the phylogenetic tree is consisted with  $G_{ST}$  values and genetic distance. The improved breeds except one line of White Leghorn (WL-4) far from the Asian native chicken populations. Ramification among improved breeds might be caused by differentiation of gene constitution in the process of improvement. The differentiation of gene constitution among improved breeds might be caused rather by random genetic drift and reduce of genetic variations during the process of improvement than by some of relationship

between economical performance and egg white protein polymorphism. Kinds and numbers of loci might change the phase of ramification. This phylogenetic tree was drawn by gene constitution of egg white protein.

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**Figure 3.** Dendrogram of 27 populations including native chicken and improved breeds drawn by neighbor-joining method

|     |                            |      |                        |
|-----|----------------------------|------|------------------------|
| VN. | Vietnam-North              | MO.  | Mongolia               |
| VS. | Vietnam-South              | MY.  | Myanmar -Yangon        |
| LN. | Laos-North                 | MM.  | Myanmar-Mandalay       |
| LC. | Laos-Central               | TS.  | Thailand-South         |
| LS. | Laos-South                 | BB.  | Boris Brown            |
| CC. | China-Chahua chicken       | IB.  | Isa Brown              |
| CG. | China-Xishuangbanna game   | WL1. | White Leghorn (WL-C36) |
| CN. | China-Xishuangbanna native | WL2. | White Leghorn (WL-C37) |
| CW. | China-Wuding chicken       | WL3. | White Leghorn (WL-S2)  |
| CY. | China-Yangbi Huang chicken | WL4. | White Leghorn (WL-S5)  |
| II. | Indonesia-Java             | WC1. | White Cornish (WC-1)   |
| IB. | Indonesia-Bali             | WC2. | White Cornish (WC-2)   |
| NW. | Nepal-West                 | WC3. | White Cornish (WC-3)   |
| NE. | Nepal-East                 |      |                        |

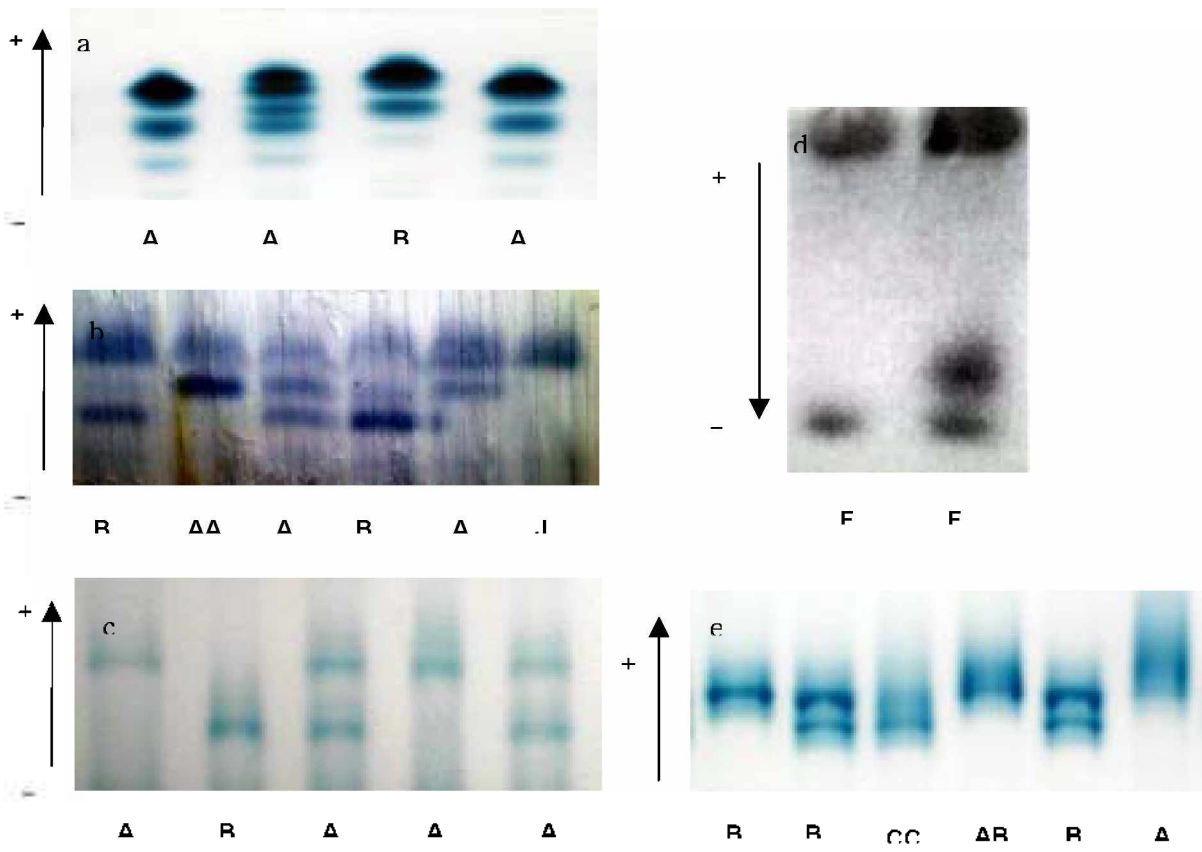
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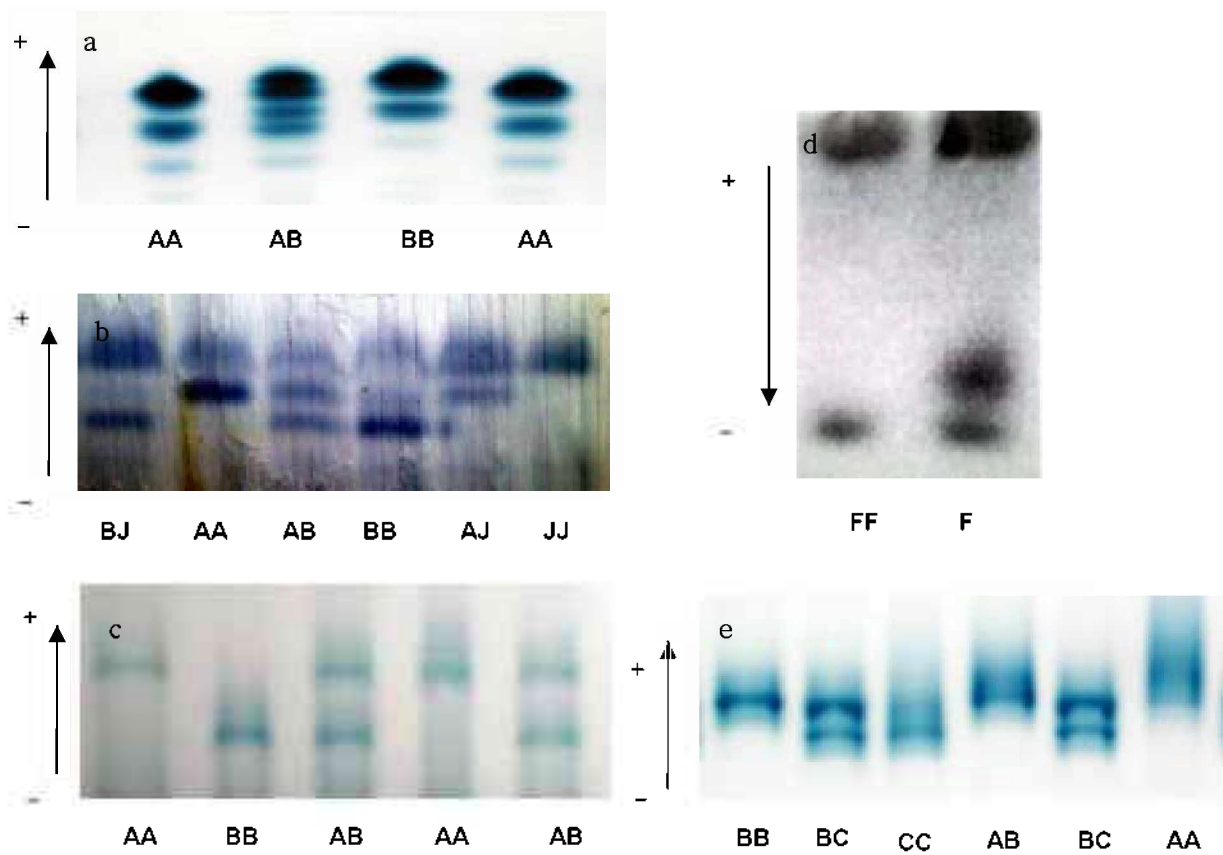
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**Figure 2.** Electrophoretic phenotypes of polymorphic egg white protein loci observed in Asian native chicken populations (a) Ovalbumin. (b) Ovoglobulin G<sub>3</sub>. (c) Ovoglobulin G<sub>2</sub>. (d) Lysozyme. (e) Ovotransferrin