SHORT COMMUNICATION

GENETIC VARIATION OF HEMOGLOBIN IN JAPANESE QUAIL

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Washburn *et al.* (1968) and Lowe *et al.* (1969) found genetic variation in minor component of hemoglobin in Japanese quail, using electrophoresis. However, they did not give symbols for those genes. In this study, the authors reexamined mode of inheritance of hemoglobin phenotype in Japanese quail, and named the hemoglobin genes, the frequencies of which in various populations were also analyzed.

Five random bred populations were used in this study. Four of five random bred populations (Toyohashi population) were introduced from a fancier in Toyohashi city to Kagoshima University during 1971 to 1974. One of five populations, Kagoshima population, has been maintained as a closed flock from 1968 in our laboratory, with a total number varying around 150 birds in each generation and the 4 generations from November 1970 to May 1972 were used in this study.

At 4 weeks of age, the blood was taken from a wing vein with a syringe containing 5% sodium citrate, and after the plasma was removed the erythrocytes were washed 4 times in physiological saline. Erythrocytes (1 volume) were hemolyzed by distilled water (10 volume); and cell debris was removed by centrifugation and the supernatant was used for analysis of hemoglobin type. Within 2 days after collection, all samples were subjected to agar gel electrophoresis. Agar gel electrophoresis was carried out using a continuous buffer system. For routine assay of hemoglobin, tris-EDTA-citric acid buffer containing 0.249 M tris (hydroxymethyl)-aminomethane, 0.008 M EDTA and 0.037 M citric acid (pH 8.6) was used for the bridge solution. For the gel preparation, the bridge buffer was diluted one sixth (pH 8.6), and 1.5 g agar (Special Agar-Noble) and 4.0 g of polyvinylpyrrolidone (Luviscol K 90 powder, molecular weight *ca.* 700,000) were dissolved in 200 ml of the diluted buffer. The electrophoresis was performed in an ice box at 5°C for about 3 hours at 12.5 mA/cm². After the electrophoresis, the gel plates were stained by the benzidine method for hemoglobin (J. DE. Grouchy 1960). Three hemoglobin phenotypes observed in this random bred population are shown in Fig. 1.

These patterns are the same as those reported by Washburn *et al.* (1968) and Lowe *et al.* (1969). Hemoglobin of Japanese quail is separated into two electrophoretic regions, major component (M) in the slow mobility region, and minor components (m_1 and m_2)

Fig. 1. Electrophoretic patterns of hemoglobin in the Japanese quail.

Table 1. Distribution of hemoglobin phenotypes in progenies from various matings

Matings		No. of matings	No. of progenys	Phenotypes of progeny			P of χ^2
Male	Female			AA	AB	BB	
AA×AA		10	85	85/85*			
AA×AB		4	31	17/15.5	14/15.5		>0.50
AA×BB		7	75		75/75		
AB×AB		17	133	35/33.25	66/66.5	32/33.25	>0.90
AB×BB		3	19		11/ 9.5	8/ 9.5	>0.25
BB×AA		3	23		23/23		
BB×AB		2	22		10/11	12/11	>0.50
BB×BB		5	42			42/42	

* Observed/expected numbers.

in the fast mobility region. In the latter, there are three phenotypes according to the combination of m_1 and m_2 . In this paper, hemoglobin phenotypes Type I (one slow moving minor band), Type II (one fast moving minor band) and Type III (both slow and fast moving minor band) that were described by Washburn *et al.* were named AA, BB and AB, respectively.

The distribution of the three phenotypes AA, AB and BB in 439 progenies from each possible kind of mating is shown in Table 1. Numbers of phenotypes in the progenies from each mating are good agreement with the expected number from Mendelian law, without exception. These cross experiment data show that m_1 and m_2 in minor hemoglobin of Japanese quail are controlled by two codominant alleles as already demonstrated by Lowe *et al.* (1969). As Lowe *et al.* (1969) didn't suggest symbol of genes controlling m_1 and m_2 , the present authors designated the gene controlling m_1 as $Hb-1^A$ and the one controlling m_2 as $Hb-1^B$. The genotypes corresponding to the phenotypes AA, AB and BB are $Hb-1^A/Hb-1^A$, $Hb-1^A/Hb-1^B$ and $Hb-1^B/Hb-1^B$, respectively.

Table 2. Phenotype and gene frequencies of Japanese quail hemoglobins in several populations

Population	No. of individuals		Number of phenotyps			Gene frequency		P of χ^2
			AA	AB	BB	<i>Hb-1^A</i>	<i>Hb-1^B</i>	
Toyohashi (1971)	78	Obs.*	77	1	0	0.99	0.01	>0.50
		Exp.**	76.4	0.6	0			
Toyohashi (1972)	87	Obs.	82	5	0	0.97	0.03	>0.95
		Exp.	81.9	5.1	0			
Toyohashi (1973)	93	Obs.	87	5	1	0.96	0.04	>0.10
		Exp.	85.7	7.1	0.2			
Toyohashi (1974)	121	Obs.	115	6	0	0.98	0.02	>0.80
		Exp.	116.3	4.7	0			
Kagoshima (1970)	99	Obs.	87	11	1	0.93	0.07	>0.50
		Exp.	85.6	12.9	0.5			

* Observed number.

** Expected number on the basis of Hardy-Weinberg's equilibrium with gene frequencies of each population.

Table 3. Changes of phenotypic and gene frequency in the successive 4 generations

Generation	Year	Month	No. of individuals		Number of phenotypes			Gene frequency		P of χ^2
					AA	AB	BB	<i>Hb-1^A</i>	<i>Hb-1^B</i>	
1	1970	November	99	Obs.*	87	11	1	0.934	0.006	>0.50
				Exp.**	86.4	12.2	0.4			
2	1971	May	84	Obs.	80	4	0	0.976	0.024	>0.99
				Exp.	80	3.9	0.1			
3	1971	November	124	Obs.	120	4	0	0.984	0.016	>0.99
				Exp.	120.1	3.9	0			
4	1972	May	160	Obs.	156	4	0	0.988	0.012	>0.99
				Exp.	156.2	3.8	0			

* Observed number.

** Expected number.

The number of phenotypes and gene frequencies of hemoglobin in five random bred populations are presented in Table 2. The observed numbers of each phenotype are in good agreement with the expected number from Hardy-Weinberg's equilibrium with gene frequencies. Phenotype AA shows markedly high frequency and phenotypes AB and BB show markedly low frequencies. Phenotype BB in three Toyohashi populations (1971, 1972 and 1974) were not found. In all random bred populations, *Hb-1^B* gene showed low frequencies (0.01-0.07). Gene frequency of *Hb-1^B* in Kagoshima population (1970) are higher than that of Toyohashi population.

Changes of phenotypic and gene frequency in the successive 4 generations from November 1970 to May 1972 of random bred Kagoshima population are shown in Table 3. In all generations the observed numbers of each phenotype are in good agreement

with the expected number from Hardy-Weinberg's equilibrium with gene frequencies. In the first generation, hatched in November 1970, 11 AB and 1 BB out of birds tested were found. After the second generation, BB was not found and the frequency of *Hb-1^B* gene tends to decrease with advanced generation. Gene frequency of *Hb-1^B* was 0.066 in the first generation and that of the fourth generation 0.012. For decrease of frequency of *Hb-1^B* gene with advanced generation, two reasons are considered. One is random drift and the another is difference of fitness among three phenotypes. Maeda *et al.* (1973) found that birds showing phenotype BB are inferior to the other phenotypes (AA and AB) in the fertility, hatchability and egg production ratio. It is likely that decrease of gene frequency of *Hb-1^B* is partly due to such differences of fitness. Perhaps, *Hb-1^B* gene is hold at a lower frequency in natural population.

LITERATURE CITED

- Grouchy, J. DE, 1960 Protéines et enzymes tissulaires-une étude par électrophorese en gel d'amidon. *Revue fr. Etud. Clin. Biol.* **5**: 286-290.
- Lowe, R. H. and K. W. Washburn, 1969 Inheritance of an abnormal hemoglobin of a random bred population of coturnix. *Poultry Sci.* **48**: 1747-1749.
- Maeda, Y., T. Hashiguchi, and M. Taketomi, 1973 Association of hemoglobin phenotypes with some economic traits in Japanese quail. *Jap. J. Zootech. Sci. Supplement* **43**: 42.
- Washburn, K. W., H. L. Marks, and R. H. Lowe, 1968 Hemoglobin types in coturnix. *Poultry Sci.* **47**: 1641-1642.