GENETIC STUDIES ON MORPHOLOGY OF ERYTHROCYTE OF QUAIL

(Coturnix coturnix japonica)

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in
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By
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Dedicated to my Grandmother

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APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY SCIENCE

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C E R T I F I C A T E

This is to certify that the work recorded in the thesis entitled "GENETIC STUDIES ON MORPHOLOGY OF ERYTHROCYTE OF QUAIL (Cutornix Cutornix Japonica)" submitted by Shri Prabal Kanti Das in partial fulfilment of the requirements for the Degree of Master of Veterinary Science in Animal Genetics and Breeding, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

(S. K. MISHRA)
Advisor

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INTRODUCTION

During last few years cotURNIX has been extensively used as a pilot animal in poultry research for short generation interval (3 to 4 generation per year) and minirum floor space requirement. Quail is also gaining popularity as table-bird for its delicious, low calorie high protein meat. Attention of the breeders is therefore draw to this field, for developing an economic meat-type quail breed.

The science of the genetics of floating body cells, spermatozoa and erythrocyte may be utilized as important tools for determining the genetic distances between the different genetic groups available which in turn will be utilized for designing different breeding programme.

Genetics of Floating Body Cells

Genetics of gametes : The concept of the genetic control on the phenotype of gametes termed as "Genetics of Gametes" was first borne by Beatty in 1961 and since then several studies have confirmed that the phenotypic characteristics of gametes are influenced by the genetics of individual from which they arise. Studies on the genetics of gametes is mostly confined to males and very little to female.

Genetics of blood cells : Erythrocyte like spermatozoa, are also free floating body cells, the chemical constituents of blood and .inal plasma being similar. More over erythrocytes

can be easily obtained in abundance. Hence the genetics of blood cells may similarly and more easily be exploited in discrimination analysis.

Studies of the genetic effects on the phenotype of erythrocytes of animals have recently been undertaken. Among the various erythrocyte attributes, the dimensional characteristics have been extensively investigated for various genetic and non-genetic causes of variations. The present work is mainly concerned with the discrimination of three lines of quail (L_1, L_2 and L_3) on the basis of dimensional characteristics of erythrocytes.

Main Object of the Study

Genetic variations (due to breeds or lines or strains) in the dimensional characteristics of nucleated erythrocyte are of value in determining.

(a) if real genetic variations exist between lines in which there is no visual difference between birds of different lines.

(b) divergence of visually different birds of different lines, and male and female irrespective of line.

(c) how far different birds of a line are genetically away from one another.

Genetic control of the dimensional - morphological characteristics of erythrocyte has received very little attention except in case of sperm cells where such studies are classed as "genetics of gametes" (Beatty, 1958, 1970). These studies have convincingly shown that the dimensional characterlstics of gametes are influenced by the genotype/genetic constitution of the individual from which they arise as well as the genome of the gametes themselves.

In blood the cells are suspended in blood plasma like spermatozoa-free floating cells in seminal plasma.

Chemical constituent of both the blood and seminal plasma are very similar (Mukherjee and Banerjee,1980). Among the different types of blood cells, or erythrocytes or red blood corpuseles are of vital importance as they carry oxygen to the various tissues of the body. The erythrocytes in mammals are non-nucleated, where as in avian species (poultry, quail, Turkey, pegion). They are nucleated. Genetic and non-genetic influences on the nucleated erythrocytes in poultry and non-nucleated erythrocytes in a few mammalian farm animals have been studied, however no work has yet been reported in Japanese quail.

Non-Nucleated Erythrocyte of mammalian farm animals

Singh (1972) studied the genetic differences in quantitative cytomorphological characteristics of the erythrocytes of cows and buffaloes and reported that the erythrocyte radius was less in Murrah buffaloes than in cattle.

Pant and Mukherjee (1974) reported significant differences in the surface area of the red blood cells (SARBC) of Jaffarabadi, Murrah, Nili and Surti breeds of buffaloes. Differences between bulls were greater than those between breeds. Body weight was found to influence the SARBC. The influence of age on SARBC appears to be insignificant.

Pant and Arora (1977) studied erythrocyte size of the Rams of four breeds of sheep, Soviet Merino, Rambouillet, Nelli, Chokla. The surface area of optically projected erythrocyte were measured and analysed. They reported that breeds of sheep contributed less than four percent (4%) of total variation, which was not significant, variation between rams was highly significant and contributed about 9% of the total variations.

Mishra and Mukherjee (1980) observed breed differences in the surface area of the erythrocyte of Barbari, Jamunapari, Black Bengal, and Beetal bucks.

The differences between breeds were less than that due to bucks within breeds. Erythrocyte size in lighter breeds (Barbari and Black Bengal) was bigger than heavier breeds (Beetal and Jamunapari).

Nucleated Avian Erythrocyte

Keller (1933) studied the cell and nuclear size in dwarf and normal breeds of chicken and reported that at hatching, the average length and width of erythrocytes of dwarf breed were considerably bigger than the breeds of normal size, whereas in adult birds, length and width of erythrocyte of dwarf breed were lesser than those of normal breeds. They therefore drew a conclusion that the size of the breed had no influence on the size of the erythrocytes in adult size.

Fchner (1939) found a considerable variations between 11 breeds of chicken, so far the size of the erythrocyte was concerned, high negative correlation between body weight and erythrocyte area was also reported. He also observed that erythrocyte area of the younger birds were considerably larger than those of the older birds.

Kitaeva (1930) observed very slight difference in the size of the erythrocytes among three European breeds of chicken namely Langshans, Brown Leghorns and Wyandhans.

Lucas and Jamroz (1961) studied the dimensional characteristics of erythrocytes in three breeds of fowl such as white leghorn, New Hampshire, and Columbian Plymouth Rock, and reported that the length and width of cytoplasm and nucleus in white leghorn fowls were considerably smaller than these of New Hamphsire and Columbians Plymouth rocks.

Kashiwabara (1964) observed the breed differences in erythrocyte dimensions of adult fowls.

Mukherjee et al. (1971) studied the erythrocyte nuclear dimensions of day old chicks of both sexes. They observed significant differences in nuclear breadth and nuclear area between the sexes. Smaller dimensions were reported in male than in female chicks. From this they concluded that males having higher oxidation rate, were smaller in erythrocyte nuclear dimensions than female chicks having lower oxidation rate.

Choh et al. (1979) studied different dimensional characteristics of erythrocytes in meat type cocks from New Hampshire and white cornish breeds. They found significant breed difference for length and area of erythrocyte nucleus.

Khan and Mukherjee (1974) reported that significant differences existed for the length, breadth and area of erythrocyte nuclei amongst six strains of White Leghorns cocks i.e., M, T, V, My, B and IVRI.

Mukherjee and Pandya (1976) using Multivariate method, studied of three characteristics (length, breadth and area) of the erythrocyte nucleus of six indigenous strains of white leghorn cocks namely My, T, M, IVRI, B and V. They observed that the difference between any pair of strains could be completely described in terms of discriminant coefficient and the magnitude of difference measured by the generalised distance. They also reported that V from T and My from all other strains could be distinguishes using erythrocyte nuclear dimensions.

Strain B could be distinguished from strains T, M & V and the latter from T and IVRI by erythrocyte nuclear dimensions.

Singh et al. (1976) reported the existance of variations between different dimensional characteristics of erythrocytes of four strain, i.e. O, P, K & N. The area of erythrocyte and that of its cytoplasm did not vary between the strains but the nuclear size was observed to vary.

Barua et al. (1981) studied the differences between sixteen sire groups over two lines of broiler chicken in the dimensional characteristics of erythrocytes.

The oval erythrocytes were measured under optical projection for length, breadth, shape (length/breadth) and area. The two lines did not differ in any of the measurements.

Chatterjee and Mukherjee (1981) reported that by using the combination of the three dimensional characteristics of the erythrocyte nucleus such as length, breadth and area of four breeds of cocks could be grouped into two groups. Australorp and White Leghorn belong to one and New Hampshire Red and Rhode Island Red belong to another group.

PART - I

MATERIALS AND METHODS

The main objective of this project was to study the variation in the dimensional characteristics of the erythrocytes between different lines of Japanese Quails (Coturnix Coturnix Japonica).

Materials and Methods :

Three lines of Japanese Quails, namely Meat line (L_3), Egg line (L_2) and German line (L_1) were used for the present study. The German line (L_1) and Meat line (L_3) were meat type strains and the Egg line (L_2) was egg type strain. These lines were originally developed and reared by Central Avian Research Institute (CARI), Izatnagar, U.P., they were maintained under uniform conditions of management and feeding at the department of Animal Genetics and Breeding, Bidhan Chandra Krishi Viswevidyalaya, Mohanpur, Nadia.

Eight adult male birds and eight adult female birds of the same age (6 weeks) were randomly selected from each line. Body weight of each of the birds was recorded separate and from each bird four blood smears were taken by puncturing the wing vein. The air dried smears were then fixed for 3 minutes, in methyl alcohol. The alcohol fixed blood smears were stained for 48 hours in freshly prepared Giemsa stain. The stain was prepared with 2 ml of Giemsa, 8 ml of

phosphate buffer (pH 6.5) and 90 ml of CO_2 free glass distilled water.

The composition of the phosphate buffer was as foll :

Composition of phosphate buffer

1.068 gm of Na_2HPO_4 in 100 ml of water

0.816 gm of KH_2PO_4 in 100 ml of water.

The granules of Na_2HPO_4 and KH_2PO_4 were dissolved in water. Then equal volume of the both compositions were mixed and the pH of the mixture was adjusted at 6.5 by the pH meter. The stained slides were rapidly washed in double distilled water, and was kept inclined to wash away the excess of water. These slides were dried over a slide warmer at 37°C for 24 hours and were mounted in DePex.

All 192 slides were coded to veil their identities and examined in a randomized order to avoid any biasness in in determining the dimensional characteristics of erythrocytes. To determine the dimensional characteristics of erythrocyte, four oval shaped erythrocyte were selected at random from each coded slides. There were three types of erythrocytes: (a) round cells with round nuclei, (b) oval cells with oval nuclei (leptochromatic) and (c) oval cells with elongated nuclei.

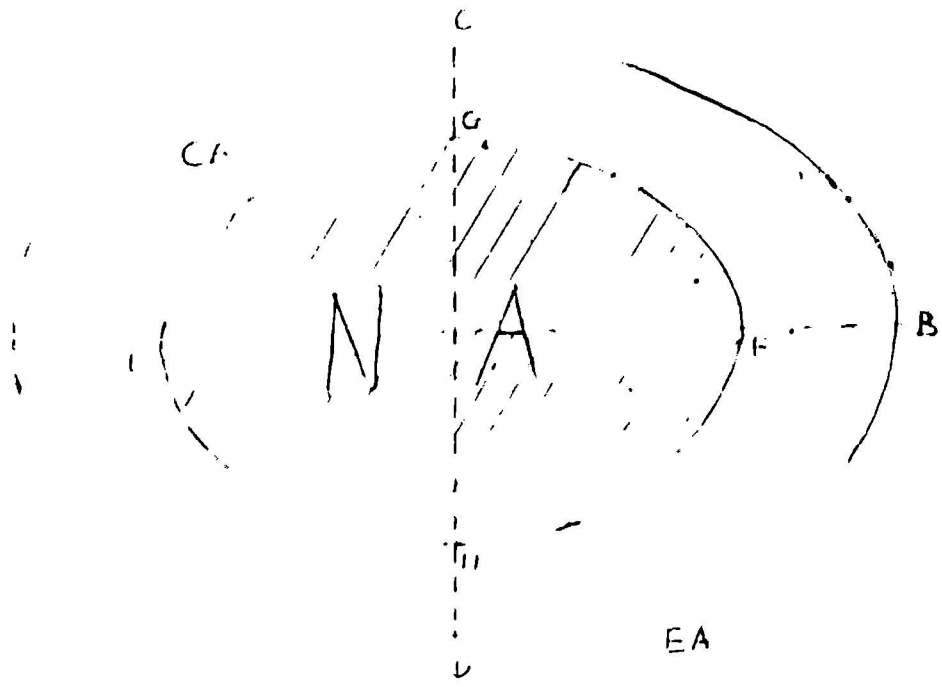


FIG. 1 SCHEMATIC DIAGRAM OF QUAIL ERYTHROCYTE WITH OVAL NUCLEUS

AB ERYTHROCYTE LENGTH

CD ERYTHROCYTE BREADTH

EF [illegible] LENGTH

GH [illegible] BREADTH

NUCLEAR AREA (NA) = [illegible] NUCLEAR AREA (NA)

CYTOPLASMIC AREA (CA) = [illegible] AREA (CA)

Camera lucida drawings of the oval shaped cytoplasm and nucleus of each erythrocyte at a magnification of 2000 were made on seperate sheets of paper.

Measurements of the maximum length, maximum breadth, and area of the cytoplasm and nucleus of erythrocyte were made on the drawings according to the method described by Pant and Mukherjee (1973). The method was as follows :

A transparent plastic scale graduated in centimeter was used to measure the erythrocyte nuclear length and breadth. To measure the maximum breadth and length, the transparent scale was moved from one end to another. When the scale reading was maximum, a line was drawn indicating the portion of maximum breadth and length and the reading was recorded.

The length of 10 μ i.e. 1 division of objective micrometer was plotted on a paper. Then the length of drawing was measured in centimeter scale and the factor was calculated in the following way.

The length of Camera Lucida drawings of one division of objective micrometer magnified on X2000, was 7.55 centimeter.

$$\therefore \quad 7.55 \text{ centimeter} = 10\,\mu$$

$$1 \text{ centimeter} = \frac{10}{7.55} = 1.3245$$

$$1 \text{ centimeter} \times 1 \text{ centimeter} = (1.3245)^2$$

$$= 1.7543 \text{ for area.}$$

The ratio of the length to breadth of erythrocyte and nucleus was taken as cytoplasmic and nuclear shape respectively. A narrow erythrocyte or nucleus would have a greater L/B ratio than broader nucleus or erythrocyte.

The area of the nucleus was determined by a planimeter graduated to 0.1 cm^2. The nuclear area was substracted from total erythrocyte area to obtain cytoplasmic area.

The erythrocyte length (EL), erythrocyte Breadth (EB) Nuclear length (NL) and Nuclear Breadth (NB), Erythrocyte area (EA), Nuclear area (NA), Cytoplasmic area (CA), Cytoplasmic shape (CS) and Nuclear shape (NS) were determined from four drawings of each slide. The average values for four drawings thus obtained for eight dimensional characters were used for further statistical analysis.

The regression of the dimensional characters on body weight of bird was calculated and necessary adjustment for the significant regression values if any on the data of respective dimensional characters were made and subjected to further analysis.

The effect of different lines, sex, and their interactions on the dimensional characters were studied using analysis of variance. The following statistical model was used.

Table I. Analysis of variance of erythrocyte (both cytoplasmic and nuclear) dimensions

Source	d.f.	M.S.
Between line (L)	(L-1) = 2	Var L
Between sex (S)	(S-1) = 1	Var S
Sex x line (L x S)	(L-1)(S-1) = 2	Var L x S
Animal within line within sex (E)	(A-1) L x S = (8-1) x 2 x 3 = 42	Var E
Between slides within animals within sex within line (C-1)	(4-1)8 x 2 x 3 = 144	Var C

The variance of animal within line within sex was tested against the variance between slides within animal within line within sex. If the animal within within line within sex was found to be significant, then the interaction of line x sex was tested against animals within line within sex. If the animal within line within was not significant, pooled

sum of square was determined by pooling animal within line within sex and error, and dividing by their sum of corresponding degree of freedom.

This pooled error measurement could be used to test variation of interaction between line and sex. If the interaction was significant, then between line and between sex variance could be tested against interaction.

If the interaction was insignificant but animal within line within sex was significant, then pooled error measurement could be estimated by adding them and dividing it with their corresponding sum of their degrees of freedom. This pooled error measurement can be used to test variation between line and between sex.

If all (animal within line within sex) were insignificant. The pooled sum of square of these two could be divided by sum of their degree of freedom, which could be used to test the main effect.

PART - I

RESULTS AND DISCUSSIONS

The means of erythrocyte nuclear and entire erythrocyte dimensional characteristics along with their standard errors, expressed in micron (μ) are presented in table-2. It is apparent from the table that means of cytoplasm area (CA) of male birds for L_1, L_2 and L_3 in μ^2 were 47.316 ± 6.861, 43.897 ± 5.394, and 49.932 ± 5.137 respectively, and 46.035 ± 3.619, 43.788 ± 4.361 and 45.615 ± 5.265 respectively in female birds. It was found in the present study that L_3 males were superior to both L_1 and L_2 and L_1 males were superior to L_2 males, whereas, L_1 female birds were superior to L_3 and L_2 and L_3 females were superior to L_2 females in respect of cytoplasm area. It is further observed that in both the sexes the L_2 birds were found to be inferior to other two lines.

From the same table it is found that the means of erythrocytes area (EA) for L_1, L_2 and L_3 lines were 61.950 ± 5.501, 59.787 ± 5.633, 64.893 ± 4.980 respectively in males and 60.194 ± 3.382, 58.015 ± 4.344 and 60.784 ± 6.361 μ^2 respectively in female birds.

The L_3 birds were found to be superior to L_1 and L_2 lines and L_1 were the L_2 birds in both the sexes.

It is apparent from the same table that the means of the nuclear area (NA) for L_1, L_2 and L_3 in male were 15.640 ±

Table 2. Mean and standard error of erythrocyte nuclear and entire erythrocyte dimensional characteristics of different lines (sex-wise)

Erythrocyte nuclear and entire erythrocyte characteristics	L_1		L_2		L_3 Y	
	Male Mean SE	Female Mean SE	Male Mean SE	Female Mean SE	Male Mean SE	Female Mean SE
Cytoplasm area (μ^2)	47.316±6.861	46.035±3.619	43.897±5.394	43.788±4.361	49.932±6.137	45.615±5.265
Erythrocyte area (μ^2)	61.950±5.501	60.194±3.382	59.787±5.633	58.015±4.344	64.893±4.930	60.784±6.361
Nuclear area (μ^2)	15.649±1.821	14.661±1.146	15.874±1.820	14.658±1.182	15.813±0.898	14.989±1.735
Erythrocyte length (μ)	10.449±0.690	10.216±0.367	9.877±0.532	9.972±0.395	10.665±0.566	10.126±0.725
Erythrocyte breadth (μ)	7.179±0.318	7.149±0.290	7.209±0.359	7.181±0.687	7.317±0.376	7.171±0.314
Nuclear length(μ)	5.348±0.389	5.301±0.195	5.352±0.272	5.207±0.269	5.435±0.252	5.324±0.429
Nuclear breadth (μ)	3.799±0.179	3.286±0.180	3.519±0.228	3.318±0.166	3.409±0.134	3.285±0.115
Erythrocyte shape	1.482±0.092	1.434±0.073	1.371±0.057	1.396±0.061	1.469±0.082	1.416±0.064
Nuclear shape	1.573±0.078	1.627±0.092	1.532±0.073	1.570±0.075	1.605±0.089	1.606±0.120

1.821, 15.874 ± 1.820 and 15.813 ± 0.698, respectively and 14.661 ± 1.146, 14.658 ± 1.182, and 14.989 ± 1.735 μ^2 respectively in female birds. In male birds, the mean nuclear area of L_2 birds was higher than L_1 and L_3, and L_3 was superior to L_1 birds. On the other hand the mean nuclear area of female birds was highest in L_3 and lowest in L_1 and L_2 was in between L_3 and L_1 birds.

The mean erythrocyte length (EL μ) for L_1, L_2 and L_3 males were observed to be 10.449 ± 0.690, 9.877 ± 0.492, and 10.665 ± 0.566 respectively and 10.216 ± 0.367, 9.972 ± 0.395 and 10.126 ± 0.725 respectively in females (Table - 2).

Mean erythrocyte length of L_2 birds were found lowest among three different lines in both the sexes.

From the table 2 it is also apparent that the mean erythrocyte breadth (EB μ) for L_1, L_2 and L_3 in male birds were 7.179 ± 0.318, 7.209 ± 0.359 and 7.317 ± 0.376 respectively and 7.148 ± 0.290, 7.181 ± 0.687 and 7.171 ± 0.337 respectively in females. Maximum erythrocyte breadth were observed in L_3 and L_2 respectively for males and females.

It is apparent from the table that the mean values of nuclear length (NL) for L_1, L_2 and L_3 were 5.348 ± 0.369,

5.332 ± 0.272, and 5.435 ± 0.252 respectively in male and 5.201 ± 0.195, 5.207 ± 0.269 and 5.324 ± 0.429 μ respectively in female birds.

In the present investigation longest erythrocyte nucleus was found in L_3 males and females.

It is apparent from the table-2 that the mean value of nuclear breadth (NB) (μ) of male birds for L_1, L_2 and L_3 were 3.799 ± 0.179, 3.519 ± 0.220 and 3.409 ± 0.134 and 3.286 ± 0.180, 3.318 ± 0.166 and 3.205 ± 0.115 respectively in female birds.

In the present study longest erythrocyte nuclear breadth was found in L_1 males and L_2 females.

Table-2 also reveals that mean erythrocyte shape (ES) for L_1, L_2 and L_3 male birds were found to be 1.482 ± 0.092, 1.371 ± 0.057 and 1.469 ± 0.082 respectively and 1.434 ± 0.073, 1.396 ± 0.061 and 1.416 ± 0.064 respectively in female birds.

Male birds of L_1 and L_3 had higher means for ES but L_2 female birds had higher mean ES over male birds.

The mean values of nuclear shape (NS) were also depicted in the same table. In male of L_1, L_2 and L_3 they were

1.573 ± 0.780, 1.532 ± 0.073 and 1.605 ± 0.089 respectively and 1.627 ± 0.092, 1.570 ± 0.075 and 1.606 ± 0.120 respectively in female. Female birds were found to be superior to males in respect of nuclear shape in L_1 and L_3, whereas L_2 birds had lowest mean value of NS for both the sexes.

Mean along with standard errors of the cytoplasm area, erythrocyte area, erythrocyte length, nuclear length, and nuclear shape, of L_1, L_2 and L_3 irrespective of sexes are presented in table-3. Different mean values of L_1, L_2 and L_3 lines were found to be 46.695 ± 3.880, 43.843 ± 3.470 and 47.774 ± 4.060 respectively for cytoplasm area, 61.072 ± 3.230, 58.931 ± 3.560 and 62.840 ± 4.040 respectively for erythrocyte area, 10.336 ± 0.391, 9.924 ± 0.330 and 10.396 ± 0.460 respectively for erythrocyte length, 5.324 ± 0.190, 5.279 ± 0.190 and 5.379 ± 0.250 respectively for nuclear length, and 1.600 ± 0.073, 1.583 ± 0.071 and 1.605 ± 0.074 respectively for nuclear shape. In the present study it was observed that when the mean for CA, EA, EL and NL were determined irrespective of sex, the L_3 line was always superior to L_1 and L_2 and L_1 was superior to L_2.

This table also revealed that the mean value of L_1, L_2 and L_3 were 1.458 ± 0.058, 1.383 ± 0.042 and 1.443 ± 0.052 respectively for erythrocyte shape; and 3.543 ± 0.180,

Table 3. Mean and standard error of erythrocyte nuclear and entire erythrocyte dimensional characteristics of different lines

Erythrocyte nuclear and entire	L_1 Mean SE	L_2 Mean SE	L_3 Mean SE
Cytoplasm area(CA)(μ^2)	46.675 ± 3.880[ab]	43.843 ± 3.470[b]	47.774 ± 4.060[a]
Erythrocyte area(EA)(μ^2)	61.072 ± 3.230	58.901 ± 3.560	62.840 ± 4.040
Nuclear area(NA)(μ^2)	15.155 ± 1.080	15.266 ± 1.090	15.401 ± 0.970
Erythrocyte length(EL)(μ)	10.333 ± 0.391[a]	9.924 ± 0.330[b]	10.396 ± 0.460[a]
Erythrocyte breadth(EB) (μ)	7.164 ± 0.220	7.195 ± 0.380	7.244 ± 0.180
Nuclear length(NL) (μ)	5.324 ± 0.190	5.279 ± 0.190	5.379 ± 0.250
Nuclear breadth(NB) (μ)	3.543 ± 0.180	3.418 ± 0.170	3.347 ± 0.120
Erythrocyte shape(ES)	1.458 ± 0.058[a]	1.383 ± 0.042[b]	1.443 ± 0.052[a]
Nuclear shape (NS)	1.600 ± 0.073	1.583 ± 0.071	1.605 ± 0.074

Means within columns bearing at least one superscript in common do not differ significantly.

3.418 ± 0.170 and 3.347 ± 0.120 respectively for nuclear breadth. Lowest mean erythrocyte shape was estimated in L_2 and lowest mean nuclear breadth was estimated in L_3.

The mean values of the L_1, L_2 and L_3 were 15.155 ± 1.080, 15.266 ± 1.090 and 15.401 ± 0.970 respectively for NA and 7.164 ± 0.220, 7.195 ± 0.380 and 7.244 ± 0.180 respectively for EB. For these characters L_3 was found to be superior to other two lines. Lower mean for NA and EB were found among L_1 birds.

It is found in this study that L_3 birds were superior to other two lines in respect of mean erythrocyte dimensional characters except in ES and NB; and the L_2 birds were inferior to other two lines in respect of mean erythrocyte dimensional characters except in NA and EB.

Means together with standard error expressed in micron (/u) of erythrocyte nuclear and entire erythrocyte dimensional characteristics of male and female over all the lines were estimated and presented in table-4.

From the table it is apparent that all the erythrocyte dimensions except NS, were larger in male than in female. The overall means together with standard error expressed in micron (/u) of erythrocyte nuclear and entire erythrocyte dimensional

Table 4. Mean and standard error of erythrocyte nuclear and entire erythrocyte dimensional characteristics of male and female of Japanese quail (over all the lines)

Trait	Sex: Male Mean	SE	Female Mean	SE
Cytoplasmic area(CA) μ^2	46.828	± 6.861	45.084	± 4.587
Erythrocyte area(EA) μ^2	62.216	± 5.768	59.740	± 4.991
Nuclear area (NA) μ^2	15.779	± 1.527[a]	14.769	± 1.375[b]
Erythrocyte length(EL) μ	10.331	± 0.685	10.105	± 0.530
Erythrocyte breadth (EB) μ	7.202	± 0.364	7.167	± 0.468
Nuclear length(NL) μ	5.378	± 0.313	5.277	± 0.319
Nuclear breadth(NB) μ	3.576	± 0.293[a]	3.296	± 0.179[b]
Erythrocyte shape(ES)	1.441	± 0.092	1.415	± 0.068
Nuclear shape (NS)	1.572	± 0.085	1.599	± 0.099

Means within a row bearing at least one superscript in common donot differ significantly.

Table 5. Overall mean with standard error of erythrocyte nuclear and entire erythrocyte dimensional characteristics of Japanese quail

Erythrocyte nuclear and entire erythrocyte characteristics	Overall mean Mean	SE
Cytoplasmic area (CA) μ^2	45.956	± 5.573
Erythrocyte area (EA) μ^2	60.977	± 5.535
Nuclear area (NA) μ^2	15.274	± 1.539
Erythrocyte length (EL) μ	10.215	± 0.624
Erythrocyte breadth (EB) μ	7.184	± 0.420
Nuclear length (NL) μ	5.328	± 0.321
Nuclear breadth (NB) μ	3.436	± 0.297
Erythrocyte shape (ES)	1.428	± 0.083
Nuclear shape (NS)	1.586	± 0.094

characteristics of Japanese quail were estimated and presented in table-5. They were 45.956 ± 5.573 μ^2, 60.977 ± 5.535 μ^2, 15.274 ± 1.539 μ^2, 10.215 ± 0.624 μ, 7.184 ± 0.420 μ, 3.436 ± 0.297 μ, 1.428 ± 0.083 and 1.586 ± 0.094 respectively for CA, EA, NA, EL, EB, NL, NB, ES and NS.

Table 6 Mean ± S.E. and C.V.% of body weight of quail

Sex of birds	Mean and S.E. (gm)	Range (gm)	C.V.%
Male	124.67 ± 4.021	67 - 150	31.60
Female	145.88 ± 7.905	72 - 199	53.09

Table-6 shows the mean along with S.E. of body weight in gms of two sexes of birds (irrespective of lines) with range and C.V.%. It is apparent from the table that the body weight of female was greater than male birds.

The regression and standard error of erythrocyte nuclear and entire erythrocyte dimensions on body weight of male and female birds was estimated and presented in table 7a and 7b respectively. No significant regression of erythrocyte nuclear and entire erythrocyte dimensions on body weight was

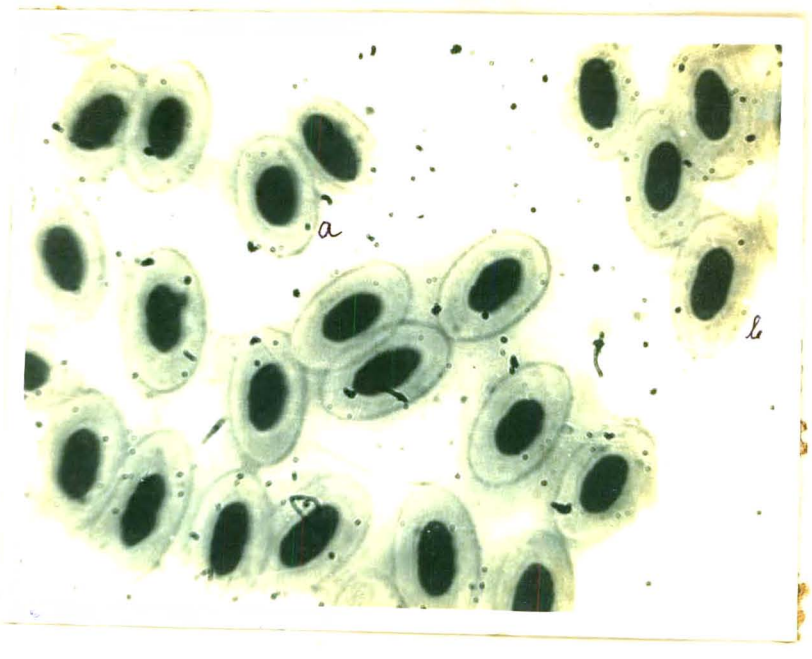


Fig.1a Microphotograph of quail erythrocytes

(a) Cell with oval nucleus (Leptochromatic) - considered

(b) Cell with elongated nucleus - (Pachychromatic) - not considered

Table 7a. Regression with S.E. of the different dimensional characteristics of nuclear and entire erythrocyte on 6th week body weight of male in different lines

Trait	Line-1 Body weight	Line-2 Body weight	Line-3 Body weight
NA (μ^2)	-0.0070172 ± 0.0416821	0.0228059 ± 0.0315774	0.0104556 ± 0.0099469
NB (μ)	0.0014569 ± 0.0029870	0.0010080 ± 0.0004600	0.0014569 ± 0.0014120
NL (μ)	0.0018540 ± 0.0031440	0.0079470 ± 0.0065956	-0.0066225 ± 0.0029270
CL (μ)	0.0172185 ± 0.0181986	0.0092715 ± 0.0118887	-0.0145700 ± 0.0067050
CB (μ)	-0.0015099 ± 0.0031580	0.0079990 ± 0.0065890	-0.0076820 ± 0.0026750
CA (μ^2)	-0.0904692 ± 0.1489946	0.0364526 ± 0.4290521	0.0403489 ± 0.2206909
CA (μ^2)	-0.1103454 ± 0.1210467	0.0593970 ± 0.0943462	0.0336825 ± 0.1787631
CS (μ)	-0.00266052± 0.0029539	-0.0004780 ± 0.0016542	-0.0007540 ± 0.0017143
NS (μ)	-0.0006776 ± 0.0021809	-0.0016116 ± 0.0015416	0.0017285 ± 0.0014771

Table 7b. Regression with S.E. of the different dimensional characteristics of nuclear and entire erythrocyte on 6th week body weight of female in different lines

Traits	Line-1	Line-2	Line-3
NA (μ^2)	0.0056500 ± 0.0072500	0.0228100 ± 0.0113800	0.0105300 ± 0.0140900
NB (μ)	-0.0000268 ± 0.0113509	0.0007862 ± 0.0012314	-0.0004630 ± 0.0012568
NL (μ)	0.0009800 ± 0.0009501	0.0039735 ± 0.003372	0.0039735 ± 0.0037230
CL (μ)	0.0004768 ± 0.0030033	0.0010596 ± 0.0058543	0.0043708 ± 0.0063046
CB (μ)	0.0026622 ± 0.0024106	-0.0029139 ± 0.0031788	0.0010344 ± 0.0026490
CA (μ^2)	0.0138400 ± 0.0250900	-0.0321000 ± 0.0684200	0.0254400 ± 0.0457900
EA (μ^2)	0.0138400 ± 0.0219300	-0.0102900 ± 0.0754300	0.0368300 ± 0.0558700
CS (μ)	-0.0004400 ± 0.0016500	-0.0009700 ± 0.0008900	0.0007030 ± 0.0008700
NS (μ)	0.0006368 ± 0.0003690	0.0007800 ± 0.0008000	0.0012600 ± 0.0008800

obtained. Hence no adjustment of data on erythrocyte nuclear and entire erythrocyte dimensions was necessary for body weights of the birds.

Though very little information were available on the dimensional characters of erythrocytes under normal conditions, it is reported in the literature that the diameter of the erythrocyte increases in pernicious anaemia and in any other abnormal conditions, where either the erythrocyte count is low. (Holman, 1952) or amount of water in plasma is more (Fiennes, 1952). The size of erythrocyte decreases in children suffering from hypochromic anaemia and in microcytic anaemia due to iron deficiency or due to inability to utilize, iron. Extreme age variation is also known to influence the erythrocyte size (Karvonen, 1954, McDonald et al., 1955). In the present study all male and female birds for three lines were choosen healthy and were of 6 weeks of age.

It is thus reasonable to assume that the variations observed for the dimensional characters in the present study were real biological differences, and not due to diseases or other abnormal conditions.

To find out whether variations existed between the lines were real or due to chance, the basic data were statistically analysed as per model gi . . in Table-1. The mean

squares for different sources of variation are presented in Table-8. The mean squares due to lines and due to sex were tested against the mean square due to line into sex. Mean square between animals within sex within line was used to test the mean square between line x sex and mean square for error component of variance was used to test the mean square between animals within sex within line.

It is apparent from the table that significant variations ($P < 0.05$) between lines were found for ES, EL and CA. Significant variations ($P < 0.01$) for NA and ($P < 0.05$) for NL were obtained.

Highly significant ($P < 0.01$) variations between animals within sex within line were found for all the dimensional characters except for nuclear shape and nuclear breadth. To test the significant difference between the means of different lines and between the sex for the traits showing significant variation. Duncan's Multiple Range Test (1955) were carried out and the result of the test are summarised in Tables-3.

Table-3 revealed that CA of L_3 was significantly greater than L_2 birds only and the L_1 birds differed significantly neither from L_2 nor from L_3 birds.

Table 8. Analysis of variance of erythrocyte dimensional characteristics

Source of variation	df	MEAN SQUARES								
		Cytoplasm area	Erythrocyte area	Nuclear area	Erythrocyte length	Erythrocyte breadth	Nuclear length	Nuclear breadth	Erythrocyte shape	Nuclear shape
Between lines	2	85.5234*	80.8672	0.3168	2.4985*	0.1567	0.0919	0.3552	0.0989*	0.1344
Between sex	1	56,4531	101.0625	15.8867**	1.3125	0.0336	0.2773	2.1402*	0.0318	0.0011
Line x sex	2	24.5000	9.5313	0.2002	0.9761	0.1066	0.0219	0.3857	0.0302	0.1463
Animal within line within sex	42	26.4695** (26.37)	26.8281** (26.0418)	1.7326** (1.6630)	0.6518** (0.6665)	0.2061** (0.2016)	0.1403** (0.1340)	0.4754	0.1691** (0.1752)	0.1441
Error	144	4.8206	3.5189	0.4029	0.0501	0.0701	0.0334	0.5688	0.0021	0.1362

* P < 0.05 ** P < 0.01

Figure in parenthesis are average M.S. for Line x sex and animal within line within sex. Used for testing the significance of between line mean squares.

It is apparent from the table-3 that the erythrocyte length (EL) of L_1 and L_3 birds were significantly larger than L_2 birds. But the difference between L_1 and L_3 was not significant.

From table-3 it is also apparent that (ES) of L_1 and L_3 birds were significantly higher than that of L_2 birds. But the differences between L_1 and L_3 birds for this character were not statistically significant.

The DMR test revealed that significant differences between the sex for NA and NB were found (Table-4). For both the traits the male birds were found to have higher dimensions.

As the body weight of male was less than female, oxygen carrying capacity of blood was more in the male than in the female. It resulted in the greater erythrocyte area (EA). erythrocyte length (EL) and erythrocyte breadth (EB) and cytoplasm area (CA) in male than in the female erythrocytes. This result is in agreement with the findings of Mehner, 1938, and Lucas and Jamroy (1961).

But the erythrocyte nucleus was greater in male than in the female. This results was not in agreement with the findings of Lucas and Jamroj (1961), Kashiwarbra (1964) and (Mukherjee et al., 1971).

A significant variation between sex was observed for NA and NB. This same result was observed by Mukherjee and Panda (1971) in male and female of 1-day old chicks in poultry.

The main observations of the dimensional characteristics of erythrocyte in permanent Giemsa preparation varied between three lines.

Variations between lines, line x sex interaction and animal within sex within line in the dimensional characteristics of erythrocytes may be due to genetic and non-genetic factors, such as age, nutrition, management and climatic factors (Khan and Mukherjee, 1974), Beatty and Sharma, 1960, Beatty, 1970; Sinha and Mukherjee, 1975; Shah et al., 1979.

In the present study nutrition and management were the same for all the birds of L_1, L_2 and L_3 and blood samples also from each birds of three lines were collected in the same day. All the birds used for the study were taken from same hatch.

The body weight also did not influence the dimension of erythrocyte as no significant regression of dimensional characters of erythrocyte on body weight were obtained. All the permanent slides and the measurements of the dimensional

characteristics of erythrocytes were done by one person (the author) by using one set of apparatus. Therefore all the possible non-genetic causes which might influence the dimensional characteristics of erythrocytes were held constant. Therefore the observed variations in the dimensional characteristics of erythrocytes were mainly due to genetic variations between lines and animal within sex within line.

In the literature no report on the dimensional characters of the erythrocyte is found in Japanese quail. However few information on these characters in poultry are available. In the variations between lines for EL, ES and CA were found. This findings are not in agreement with respect of Khan and Mukherjee (1974), Barua and Pant (1981) and Singh and Mukherjee (1976).

This difference in the findings with other workers might be due to infact that the type variations of erythrocytes dimensional characters of quail are not similar to that of poultry. However, before drawing any inference, it is felt that further study on these characters in quail is needed.

PART - II

MULTIVARIATE ANALYSIS IN THE ERYTHROCYTE DIMENSIONAL CHARACTERISTICS OF DIFFERENT LINES OF JAPANESE QUAIL

PART - II

MATERIALS AND METHODS

In the previous chapter it has been observed that differences among lines exist in respect of the single dimensional characteristics of erythrocyte of three lines of Japanese quail. Now the lines can be discriminated by taking more than one of the single dimensional characteristics through multivariate technique of analysis. Fisher's discriminant functions i.e. the linear combination of single characteristics that gives maximum discrimination between lines can be calculated.

The objectives of study mentioned in this chapter were therefore,

(i) to discriminate between the genetic groups (lines) by calculating the discriminant functions,

(ii) to determine the relative closeness of the lines from one another, on the basis of the genetic differences in the basis of the genetic differences in the erythrocyte dimensions.

Materials and methods :

The basic data on the dimensional characteristics of the erythrocyte from 24 males and 24 females to 3 different lines of the previous study were used. Only data on the erythrocyte length, erythrocyte shape and cytoplasm area which

showed significant variations between line were utilized in the present study.

Statistical Methodology :

To calculate the discriminant function owing to discriminate between lines by using the various dimensional characteristics of erythrocyte namely, EL, ES, CA it was necessary to generate sum of squares (SS) and sum of products (SP) matrices between lines and between line x sex and animal within line within sex. The sum of squares for the three sources of variations were already calculated. Here sum of square of both line x sex and animal within sex within line were added up to get total sum of square which will be considered as error sum of square (C_{ij}). The method of analysis was the same as mentioned in the previous chapter. The sum of products were calculated as per Table 8a and b which was same as mentioned in the previous chapter.

All the differences between lines were tested by forming a combination of (i) three linear discriminant functions for three erythrocyte dimensions.

Table 9a Analysis of covariance of erythrocyte (cytoplasmic and nuclear) dimensions

Source of variation	d.f.	S.P.	
Between line (B)	L-1 = 2	B_{ij}	
Between sex (S)	S-1 = 1	S_{ij}	
Sex x line (L x S)	(L-1)(S-1) L x S = 2	L_{ij}	C_{ij}
Animal within (A) sex within line	(A-1)L x S = 42	A_{ij}	
Between slides within animal(E) within sex (S) within line	(S-1)L x S x A E = 144	E_{ij}	

Table 9b. Analysis of variance of erythrocyte (cytoplasmic and nuclear) dimensions

Source of variation	d.f.	S.S.	
Between line (B)	L - 1 = 2	B_{ij}	
Between sex (S)	S - 1 = 1	S_{ij}	
Sex x Line (LS)	(L-1) (S-1) = 2	L_{ij}	C_{ij} ..
Animal within sex within (A) line	(A-1)L x S = 42	A_{ij}	
Between slides within animal, within sex within line (S)	(S-1)L x S x A = 144	E_{ij}	

Since the means of the erythrocytes cytoplasms differed, they were assigned equal and proportionate weights (Goulden, 1956). The weights assigned were $a_j = (1/\bar{x}_j) \times 100$. where, j = 1 to 3 the number of dimensional characteristics studied.

It was apparent that the dimensional characteristics with lower mean ($\bar{x}_j$) would have a higher assigned weight then the erythrocyte dimension with higher mean the discriminant coefficient (b_j) for each combination of the dimensional characteristics was determined from the following equation:

$$\sum_{j=1}^{p} b_j \cdot B_{ij} = \sum_{j=1}^{p} a_j \cdot G_{ij} \qquad \text{(Rao, 1952)} \qquad ..$$

In the equation the genotypic sum of squares and products (C_{ij}), all unknown parameter, was estimated from

$$G_{ij} \sim B_{ij} - \frac{(L-1) = 2}{(L-1)(G-1)4(A-1)5xS = 42} \times C_{ij} \text{ (Rao, 1952)}$$

$$G_{ij} \sim B_{ij} - 0.0454545 \times C_{ij}$$

The discriminant functions were used to obtain the discriminant scores (Z) for each line.

The difference between the discriminant scores between a line pair was the Generalized or (Mahalanobis)

Distance Function D^2. Square root of the Generalized Distance Function was the Distance D.

The D^2 values were tested for significance by Hotelling's T^2-test (Snedecor and Cochran, 1960).

Hotellings (1940) T^2 test. The equation being

$$T^2 = \frac{n_1 \cdot n_2}{n_1 + n_2} (adx + bdy + cdz)^2$$

where, a, b, c were determined by

$$\begin{matrix} a \\ b \\ c \end{matrix} = \begin{matrix} \Sigma x^2 & + \Sigma xy & + \Sigma xz \\ \Sigma xy & + \Sigma y^2 & + \Sigma yz \\ \Sigma xz & + \Sigma yz & + \Sigma z^2 \end{matrix}^{-1} \begin{matrix} dx \\ dy \\ dz \end{matrix} \quad \cdot\cdot$$

$$\begin{matrix} a \\ b \\ c \end{matrix} \begin{matrix} \Sigma x^2 & + \Sigma xy & + \Sigma xz \\ \Sigma xy & \Sigma y^2 & + \Sigma yz \\ \Sigma xz & \Sigma yz & + \Sigma z^2 \end{matrix} = \begin{matrix} dx \\ dy \\ dz \end{matrix}$$

dx, dy, dz, the differences between means of respective traits of the two genetic groups.

The ^{2} value was tested by the analysis of variance

Source of variation	d.f.	M.S.	F
Between genetic groups	p	T^2/p	
Within genetic groups	$(n_1 + n_2 - p - 1)$	$\frac{(adx+bdy+cdz)}{(n_1+n_2-p-1)}$	

where, p = no. of traits studied.

The misclassification probability (MCP) denoted by ( ) expressed in percentage was equal to $(1 - \Phi/2) \times 100$ where Φ was the normal distribution function. The D^2 values between line pairs were used to study the relative closeness of the lines (Rao, 1952).

PART - II

RESULTS AND DISCUSSION

The generalized sum of squares (SS) and sum of products (SP) between lines, (B_{ij}) between line x sex and animal within sex within line (C_{ij}) and between genotype (C_{ij}) for the erythrocyte dimensions such as CA, ES, EL are presented in Table 10, 11 and 12. The highest phenotypic variance was found in CA, followed by EL and ES. The value of variances are represented diagonally in the Table 10.

Table 13 represents the inverse of the phenotypic matrix $(E_{ij})^{-1}$. Discriminant coefficient b_j, discriminant functions ($b_j \times \bar{X}_{ij}$) and discriminant score, (S) for each line using erythrocyte dimensional characteristics are given in Table 14. In all these lines CL contributing maximum towards the D values. Discriminant coefficient and D^2-values, Distance (D) and Misclassification probabilities (M.C.P.) in percentage between line pairs are presented in Table 15. The D^2 values for all line pairs were tested by Hotelling's T^2 test and were found to be highly significant, indicating that the combination of three dimensional characteristics differed between each line pair in which D increased, the M.C.P. decreased. The D values for the distinguishable were 8.80 for L_1 and L_2 line pairs and 9.98 for L_1 and L_3 line pairs and 4.69 for L_2 and L_3 line pair.

The relative contribution of each character towards the D^2-values between line pair are presented in Table 15. CA contributes maximum towards D^2-values followed by EL and ES.

Using the dimensional characteristics of erythrocyte the D^2-values of L_1, L_2 and L_3 are distinctly separated from each other. Hence no cluster will be formed among three lines.

Multivariate technique of analysis of the dimensional characteristics of CA, EL and ES was considered, to differentiate the three lines of Japanese Quail. This technique was used by Mukherjee and Pandya (1976) used spermatozoan and erythrocyte nuclear dimensions seperately to discriminate strains of white leghorn cocks.

These results did not agree with those reported by Mukherjee and Pandya (1976). They reported that erythrocyte NL, NB & NA were the important dimensional characteristics to discriminate the strains. In the present study ES, EL and CA were found to be important dimensional characteristics to discriminate the lines of Japanese quails.

Table 10. Between line Phenotypic Matrix (B_{ij})

	CA (X_1)	ES (X_2)	EL (X_3)
CA	171.046900	5.173843	28.904480
ES	5.473843	0.1977234	0.942795
EL	28.904480	0.942795	4.997071

Table 11. Between line x sex and Animal within sex within line (C_{ij}) Matrix

	CA	ES	EL
CA	1160.719000	15.084619	179.726390
ES	15.084619	0.770660	2.019033
EL	179.726390	2.019033	29.329099

Table 12. Genotype matrix (G_{ij})

	CA	ES	ES	e_j
CA	118.2869455	4.4881785	20.7350986	3.8056
ES	4.4881785	0.7386304	0.8510208	70.0172
EL	20.7350986	0.8510208	3.6639301	12.9671

Table 13. Inverse of the phenotypic matrix $[B_{ij}]^{-1}$

	CA	ES	EL	$[G_{ij}][a_j]$
CA	-12.1267629	-170.8118270	102.3716318	1033.2766
ES	-170.8118270	-2355.5878880	1432.4518350	79.8323
EL	102.3716318	1432.4518350	-862.2065212	186.0061

Table 14. Discriminant coefficient and discriminant score (Z) for each line

b_j	-7124.8534	-98103.0835	59758.4639	Z-value
Lines	CA	SS	SL	
L-1	-189566.8420	-143065.2960	466205.6561	133573.5181
L-2	-178063.4456	-135751.3681	447310.8067	133495.9930
L-3	-194025.1470	-141522.0858	469021.1463	133473.9127

Table 15. Discriminant coefficients and D^2 values, Distance (D) and Misclassification probabilities (M.C.P.) in percentage between line pairs

Line pairs	CA	ES	EL	D^2-value	D (Distance)	M.C.P.%
L-1/L-2	-11503.3964	-7313.9279	18894.8494	77.5251	8.80**	0.00
L-1/L-3	4458.3058	-1543.2102	-2815.4902	99.6054	9.98**	0.00
L-2/L-3	15961.7022	5770.7177	-21710.3396	22.803	4.69**	0.96

** P $\angle$ 0.01

Inter cluster average D^2-values

	L_2	L_3
L_1	77.53	99.61
L_2	-	22.08

Inter cluster average D-values

	L_2	L_3
L_1	8.80	9.98
L_2	-	4.69

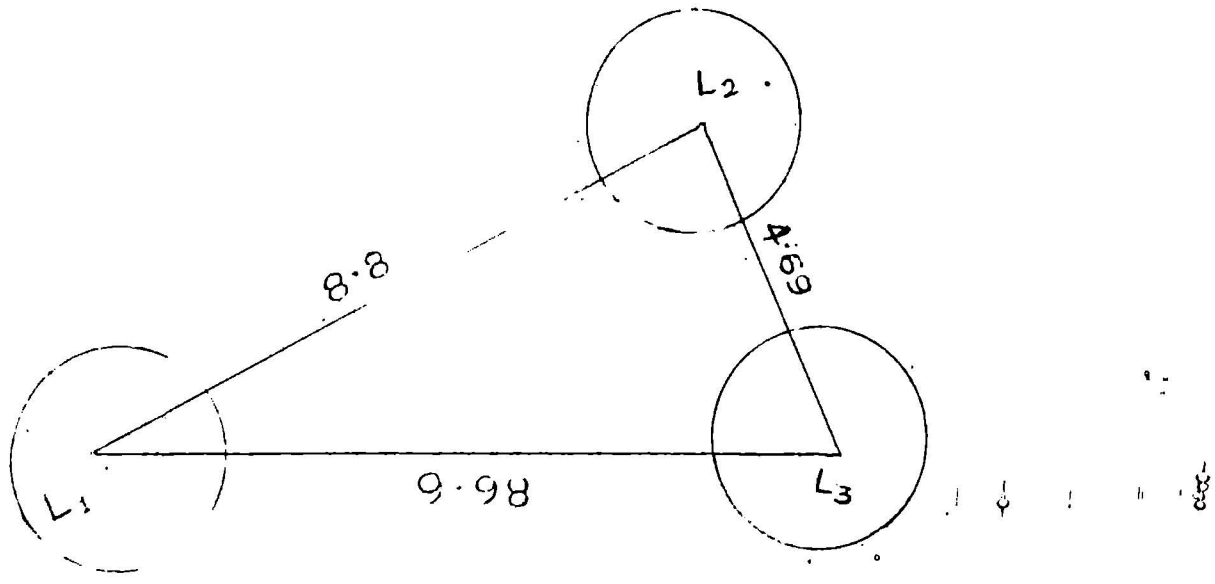
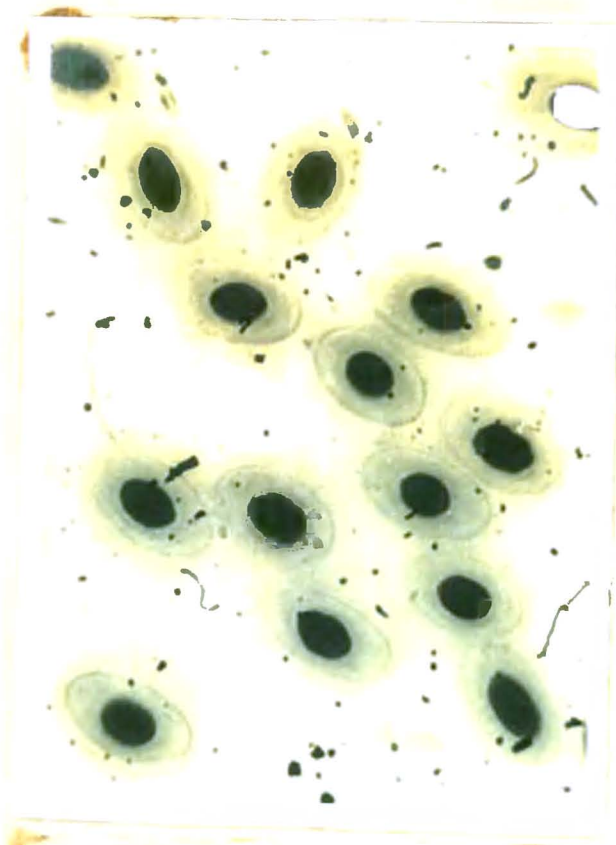
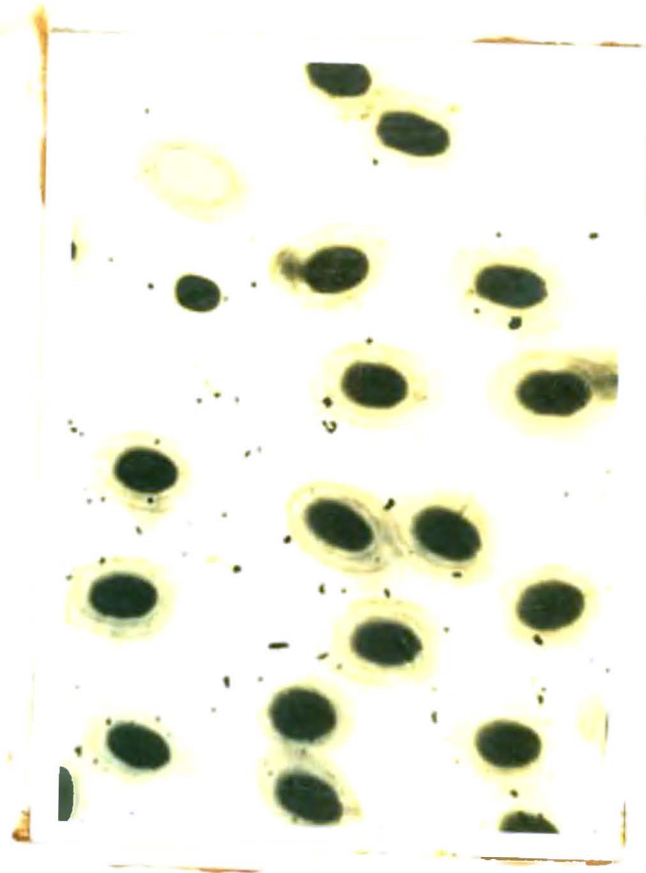


Fig 2 CONFIGURATION OF THE THREE GROUPS AND THEIR MUTUAL RELATIONSHIPS BASED ON THE ERYTHROCYTE & CYTOPLASM DIMENSIONS.
(SCALE 1D = 1 c.m.)

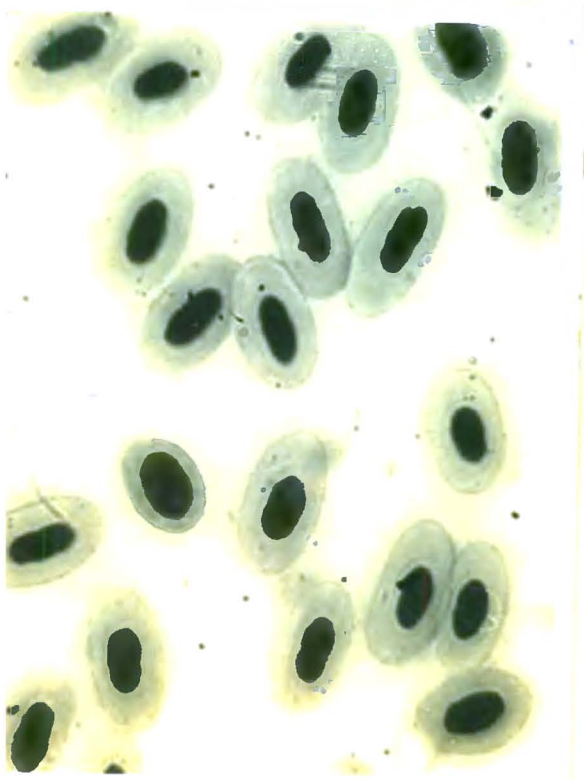
(D 8·8, 9·98, 4·69)



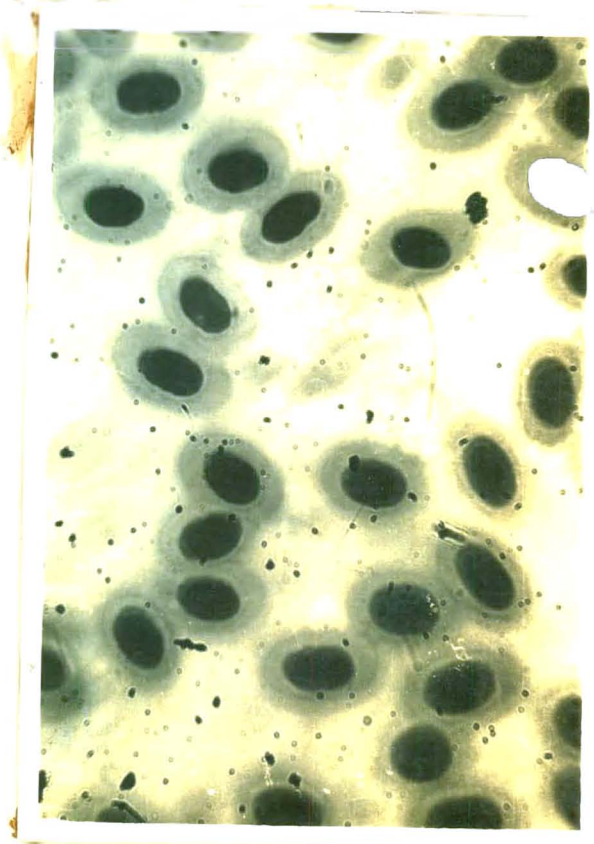
Line-1
German line (Male)



Line-1
German line (Female)

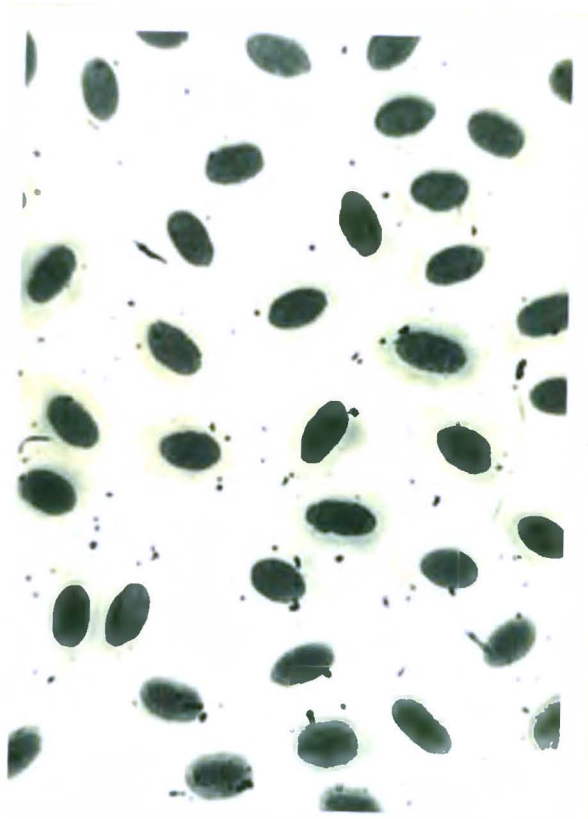


Line-2
Egg line (male)

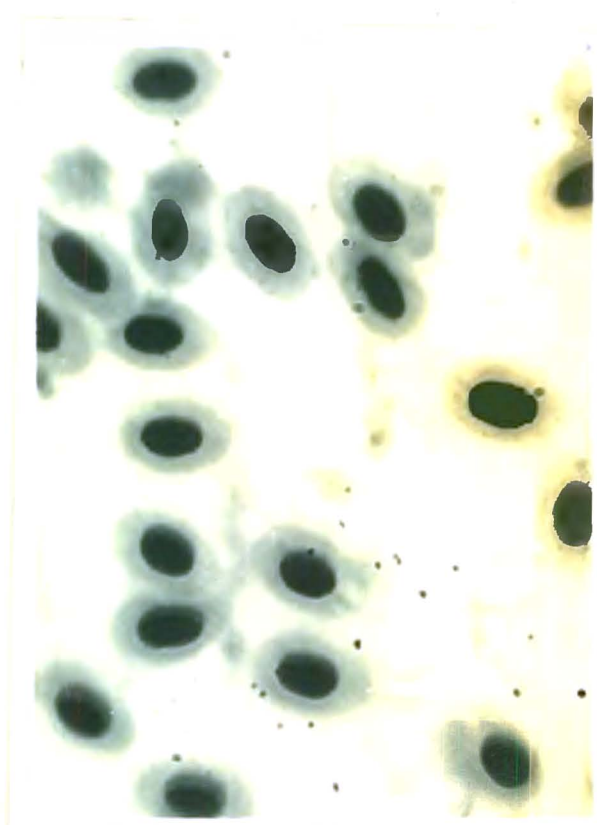


Line-2
Egg line (female)

Fig.2a Microphotographs of quail erythrocytes of the three lines. line-1 (German line - Male & Female), line-2 (Egg line - Male & Female)



Line-3
Meat line (male)



Line-3
Meat line (female)

Fig.2a Microphotographs of quail erythrocytes of the three lines. line-3 (Meat line - male & female)

SUMMARY AND CONCLUSION

The dimensional characteristics of erythrocyte in permanent geimsa stain preparations from three lines namely German line (L_1), egg line (L_2), meat line (L_3) maintained under the same dietary and managerial conditions, and of same age were prepared.

The following results were obtained -

i) Line difference are found in the cytoplasm area, and erythrocyte length and erythrocyte shape.

ii) Between animal within lines within sex except nuclear breadth and nuclear shape all the dimensional characteristics of erythrocyte varied.

iii) Between line x sex, all the dimensional characteristics of erythrocyte did not vary.

iv) The dimensional characteristics of erythrocyte length, area and cytoplasm area differentially discriminated the three lines.

The multivariate method of analysis was applied by using three dimensional characteristics namely, Erythrocyte length, Erythrocyte shape, and Cytoplasm area which varied significantly between three lines namely German line, egg line and meat line to discriminate and to determine the nearness of these lines.

The following results were obtained -

i) By using combination of three dimensional characteristics of erythrocyte namely Erythrocyte length, erythrocyte shape, and cytoplasm area the lines could not be grouped. These three lines were found to be distinctly separated from each other.

ii) It was observed that the difference between any two lines could be completely described in terms of discriminant coefficients and the magnitude of the difference measured by the Generalized Distance Function (D^2).

iii) It was also observed that meat line and German lines and egg line were genetically seperated from each other, of which egg line and meat line are comparatively closer than their distance from German line.

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