

COMPARATIVE RESEARCH CONCERNING HARDY–WEINBERG EQUILIBRIUM IN FOUR STATISTICAL SWINE POPULATIONS

Nicoleta DEFTA, Răzvan POPA, Tomița DRĂGOTOIU, Izabela OPREA,
Paula POȘAN, Dana POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd,
District 1, Bucharest, Romania

Corresponding author email: poparasvan@yahoo.co.uk

Abstract

The state of genetic balance in one or more loci, no matter if it concerns the loci involved in genetic determinism of quality or quantity traits, describes an ideal state, being influenced by natural selection, non-random mating, gene flow and genetic drift. The Hardy-Weinberg equilibrium trial presents a significant importance because any deviation from it reveals the existence of some disturbing, restrictive factors. Thus, in this direction, there were researched four swine statistical livestock, taking into account the aggregate genotype derived from prealbumins, transferrins and serum amylases. The significant distribution of the individuals which present the genotypical combination $Pa^bPa^b/Tj^bTj^b/Am^bAm^b$ (Landrace - 39.5%; Large White - 23.7%; Synthetic Breed P 2000 - 22.7%; Synthetic 345 - 31.9%) was characteristic of the four researched samples. Out of the 27 possible combinations in the three loci, there weren't identified individuals with the aggregate genotypes in any sample: $Pa^aPa^a/Tj^aTj^a/Am^aAm^a$; $Pa^aPa^a/Tj^aTj^a/Am^aAm^b$; $Pa^aPa^a/Tj^aTj^b/Am^aAm^a$; $Pa^aPa^a/Tj^bTj^b/Am^aAm^a$; $Pa^aPa^b/Tj^aTj^a/Am^aAm^a$; $Pa^aPa^b/Tj^aTj^b/Am^aAm^a$; $Pa^aPa^b/Tj^bTj^b/Am^aAm^a$. In order to estimate the balance state, it was calculated the determinant of the gamete matrix. The result of the genetic state Hardy Weinberg trial, simultaneously for the three loci ($g_{11}g_{33}g_{17}g_{39} = g_{31}g_{13}g_{37}g_{19}$), emphasized the fact that none of the livestock is in genetic balance, as it follows: Landrace (0.0000215 ± 0.0000067), Large White (0.000039 ± 0.000041), Synthetic Breed P 2000 (0.000055 ± 0.000025), Synthetic Breed 345 (0.000004 ± 0.000030). This shortage of balance was caused by an indirect selection, with a higher coefficient of some genotypic combinations in comparison with the others.

Key words: Hardy-Weinberg equilibrium, gamete matrix, genotypic matrix, pig populations.

INTRODUCTION

Protein polymorphism manifests itself from birth and, with some exceptions, it is constantly maintained throughout the life of the animal (Bacila V. et al., 2011). It is possible to use it as an early selection tool for high-value breeders since some protein fractions can be correlated with some economic traits (Drăgotoiu T., 2005).

Also, the importance of knowing proteins genetic determinism (protein fractions) lies in the application of information in this field, in the following directions:

- Clarification of cases of uncertain origin since biochemical systems provide information on the specific genetic material of each individual (Rebedea, 1991);
- Establishing paternity (Rebedea, 1992);
- Decoding the genetic mechanisms responsible for the existence of polymorphism

at the level of biochemical structures (Juneja, 1988);

- Establishing phylogeny and kinship among breeds, based on the frequencies of genes that determine different biochemical structures (Defeta, 2001), structures characteristic to certain breeds and population. "Changes in allele frequencies over time can indicate that genetic drift is occurring or that new mutations have been introduced into the population" (www.nature.com).
- Being major genes, their manifestation is discontinuous, thus, they can be used as marker genes, establishing correlations among them and quantitative traits loci (QTL). "Quantitative trait locus (QTL) analysis is a statistical method that links two types of information - phenotypic data (trait measurements) and genotypic data (usually molecular markers) - in an attempt to explain the genetic basis of variation in complex traits" (Miles, 2008).

MATERIALS AND METHODS

a. Material and laboratory methods

To determine the types of serum proteins, i.e. prealbumins, transferins and amylases, there were taken blood samples from 290 heads of the following breeds: Landrace (76), Great White (76) and synthetic lines P 2000 (66) and 345 (72).

The subjects that constituted the source of blood sampling were chosen at random.

The vertical electrophoresis technique was used to determine the types of *transferins* and *prealbumins* in the study individuals. (Smithies, 1955; Costache, 2004), using polyacrylamide as migration support.

| | | | |
|-------------|-------------|-------------|-------------|
| $A_1B_1C_1$ | $A_1B_1C_2$ | $A_1B_2C_1$ | $A_1B_2C_2$ |
| $A_2B_1C_1$ | $A_2B_1C_2$ | $A_2B_2C_1$ | $A_2B_2C_2$ |

Knowing the frequencies of the genotype categories, the population's gamet background was also determined, knowing that, for example, the category of gametes of type $A_1B_1C_1$ will represent 100% of the gametes produced by genotype $A_1A_1/B_1B_1/C_1C_1$; 50% out of the gametes produced by

To emphasize the types of *serum amylases*, electrophoresis in starch gel was used as a working method in a discontinuous system of swabs (Meriaux, 1992).

b. Data processing methods

For the three loci, the number of genotypic combinations which may result is 27.

Thus, the categories frequencies of aggregate genotypes were organized into a matrix, with three rows and nine columns.

Considering account simultaneously the three loci, each one, with simple allelism, can result in eight categories of gametes, the frequency of which is organized in a matrix of the type:

$$\text{respectively } g = \begin{bmatrix} g_{11} & g_{13} & g_{17} & g_{19} \\ g_{31} & g_{33} & g_{37} & g_{39} \end{bmatrix}$$

$A_1A_1/B_1B_1/C_1C_2$, $A_1A_1/B_1B_2/C_1C_1$ and $A_1A_2/B_1B_1/C_1C_1$ genotypes; 25% out of the gametes produced by the $A_1A_1/B_1B_2/C_1C_2$ genotypes; $A_1A_2/B_1B_1/C_1C_2$ and $A_1A_2/B_1B_2/C_1C_1$ and 12.5% out of the gametes produced by the genotype $A_1A_2/B_1B_2/C_1C_2$.

$$\begin{aligned} A_1B_1C_1 &= g_{11} = G_{11} + \frac{1}{2}(G_{12} + G_{14} + G_{21}) + \frac{1}{4}(G_{15} + G_{22} + G_{24}) + \frac{1}{8}G_{25} \\ A_1B_1C_2 &= g_{13} = G_{13} + \frac{1}{2}(G_{12} + G_{16} + G_{23}) + \frac{1}{4}(G_{15} + G_{22} + G_{26}) + \frac{1}{8}G_{25} \\ A_1B_2C_1 &= g_{17} = G_{17} + \frac{1}{2}(G_{14} + G_{18} + G_{27}) + \frac{1}{4}(G_{15} + G_{24} + G_{28}) + \frac{1}{8}G_{25} \\ A_1B_2C_2 &= g_{19} = G_{19} + \frac{1}{2}(G_{16} + G_{18} + G_{29}) + \frac{1}{4}(G_{15} + G_{26} + G_{28}) + \frac{1}{8}G_{25} \\ A_2B_1C_1 &= g_{31} = G_{31} + \frac{1}{2}(G_{21} + G_{32} + G_{34}) + \frac{1}{4}(G_{22} + G_{24} + G_{35}) + \frac{1}{8}G_{25} \\ A_2B_1C_2 &= g_{33} = G_{33} + \frac{1}{2}(G_{23} + G_{32} + G_{36}) + \frac{1}{4}(G_{22} + G_{26} + G_{35}) + \frac{1}{8}G_{25} \\ A_2B_2C_1 &= g_{37} = G_{37} + \frac{1}{2}(G_{27} + G_{34} + G_{38}) + \frac{1}{4}(G_{24} + G_{28} + G_{35}) + \frac{1}{8}G_{25} \\ A_2B_2C_2 &= g_{39} = G_{39} + \frac{1}{2}(G_{29} + G_{36} + G_{38}) + \frac{1}{4}(G_{26} + G_{28} + G_{35}) + \frac{1}{8}G_{25} \end{aligned}$$

Appropriate situation for three places

| | | | | | | |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| $A_1A_1B_1B_1C_1C_1$ | $A_1A_1B_1B_1C_1C_2$ | $A_1A_1B_1B_2C_1C_1$ | $A_1A_1B_1B_2C_1C_2$ | $A_1A_1B_2B_2C_1C_1$ | $A_1A_1B_2B_2C_1C_2$ | $A_1A_1B_2B_2C_2C_2$ |
| $p^2r^2t^2$ | p^2r^2tu | p^2rst^2 | p^2rstu | $p^2s^2t^2$ | p^2s^2tu | $p^2s^2u^2$ |
| $A_1A_2B_1B_1C_1C_1$ | $A_1A_2B_1B_1C_1C_2$ | $A_1A_2B_1B_2C_1C_1$ | $A_1A_2B_1B_2C_1C_2$ | $A_1A_2B_2B_2C_1C_1$ | $A_1A_2B_2B_2C_1C_2$ | $A_1A_2B_2B_2C_2C_2$ |
| pqr^2t^2 | pqr^2tu | $pqrst^2$ | $pqrst$ | pqs^2t^2 | pqs^2tu | pqs^2u^2 |
| $A_2A_2B_1B_1C_1C_1$ | $A_2A_2B_1B_1C_1C_2$ | $A_2A_2B_1B_2C_1C_1$ | $A_2A_2B_1B_2C_1C_2$ | $A_2A_2B_2B_2C_1C_1$ | $A_2A_2B_2B_2C_1C_2$ | $A_2A_2B_2B_2C_2C_2$ |
| $q^2r^2t^2$ | q^2r^2tu | q^2rst^2 | q^2rstu | $q^2s^2t^2$ | q^2s^2tu | $q^2s^2u^2$ |

where, p= the frequency of the A₁ gene; respectively Pa^A

q= the frequency of the A₂ gene; respectively Pa^B

r= the frequency of the B₁ gene; respectively Tf^A

s= the frequency of the B₂ gene; respectively Tf^B

t= the frequency of the C₁ gene; respectively Am^A

u= the frequency of the C₂ gene; respectively Am^B

and the genotypic matrix

$$G = \begin{bmatrix} G_{11} & G_{12} & G_{13} & G_{14} & G_{15} & G_{16} & G_{17} & G_{18} & G_{19} \\ G_{21} & G_{22} & G_{23} & G_{24} & G_{25} & G_{26} & G_{27} & G_{28} & G_{29} \\ G_{31} & G_{32} & G_{33} & G_{34} & G_{35} & G_{36} & G_{37} & G_{38} & G_{39} \end{bmatrix}$$

For three loci, the gametes frequency is the following: *p_{rt}* for A₁B₁C₁, *p_{ru}* for A₁B₁C₂, *p_{st}* for A₁B₂C₁, *p_{su}* for A₁B₂C₂, *q_{rt}* for A₂B₁C₁, *q_{ru}* for A₂B₁C₂, *q_{st}* for A₂B₂C₁, *q_{su}* for

A₂B₂C₂. Concerning the situation coming from three loci, the equilibrium condition is reached when the genetic matrix:

$$g = \begin{bmatrix} g_{11} & g_{13} & g_{17} & g_{19} \\ g_{31} & g_{33} & g_{37} & g_{39} \end{bmatrix} \text{ achieves the equality } g_{11} g_{33} g_{17} g_{39} = g_{31} g_{13} g_{37} g_{19}$$

RESULTS AND DISCUSSIONS

Comparative analysis of the four samples

With the methods described above, there were

identified the genotypes characteristic of the four study swine population (Table 1).

Table 1. Genetical structure of the four samples according to aggregate genotypes of prealbumins, transferins and serum amylases

| Genotypical combination | Breed | | | | | | | |
|---|----------|------|-------------|------|---------|------|--------|------|
| | Landrace | | Great White | | LP 2000 | | LS 345 | |
| | n | % | n | % | n | % | n | % |
| Pa ^A Pa ^A /Tf ^A Tf ^A /Am ^A Am ^A | - | 0 | - | 0 | - | 0 | - | 0 |
| Pa ^A Pa ^A /Tf ^A Tf ^A /Am ^A Am ^B | - | 0 | - | 0 | - | 0 | - | 0 |
| Pa ^A Pa ^A /Tf ^A Tf ^A /Am ^B Am ^B | - | 0 | - | 0 | 1 | 1.5 | - | 0 |
| Pa ^A Pa ^A /Tf ^A Tf ^B /Am ^A Am ^A | - | 0 | - | 0 | - | 0 | - | 0 |
| Pa ^A Pa ^A /Tf ^A Tf ^B /Am ^A Am ^B | 2 | 2.6 | 2 | 2.6 | - | 0 | 1 | 1.4 |
| Pa ^A Pa ^A /Tf ^A Tf ^B /Am ^B Am ^B | - | 0 | - | 0 | 4 | 6.1 | 2 | 2.8 |
| Pa ^A Pa ^A /Tf ^B Tf ^B /Am ^A Am ^A | - | 0 | - | 0 | - | 0 | - | 0 |
| Pa ^A Pa ^A /Tf ^B Tf ^B /Am ^A Am ^B | - | 0 | - | 0 | - | 0 | 1 | 1.4 |
| Pa ^A Pa ^A /Tf ^B Tf ^B /Am ^B Am ^B | 3 | 3.9 | - | 0 | 8 | 12.1 | 5 | 6.9 |
| Pa ^A Pa ^B /Tf ^A Tf ^A /Am ^A Am ^A | - | 0 | - | 0 | - | 0 | - | 0 |
| Pa ^A Pa ^B /Tf ^A Tf ^A /Am ^A Am ^B | - | 0 | 4 | 5.3 | 1 | 1.5 | - | 0 |
| Pa ^A Pa ^B /Tf ^A Tf ^A /Am ^B Am ^B | - | 0 | 1 | 1.3 | - | 0 | - | 0 |
| Pa ^A Pa ^B /Tf ^A Tf ^B /Am ^A Am ^A | - | 0 | - | 0 | - | 0 | - | 0 |
| Pa ^A Pa ^B /Tf ^A Tf ^B /Am ^A Am ^B | 2 | 2.6 | 3 | 4 | 4 | 6.1 | - | 0 |
| Pa ^A Pa ^B /Tf ^A Tf ^B /Am ^B Am ^B | 3 | 4 | 3 | 3.9 | - | 0 | 6 | 8.3 |
| Pa ^A Pa ^B /Tf ^B Tf ^B /Am ^A Am ^A | 3 | 4 | 1 | 1.3 | 5 | 7.6 | 1 | 1.4 |
| Pa ^A Pa ^B /Tf ^B Tf ^B /Am ^A Am ^B | 1 | 1.3 | 5 | 6.6 | 9 | 13.6 | 2 | 2.8 |
| Pa ^A Pa ^B /Tf ^B Tf ^B /Am ^B Am ^B | 9 | 11.8 | 10 | 13.2 | 2 | 3 | 12 | 16.7 |
| Pa ^B Pa ^B /Tf ^A Tf ^A /Am ^A Am ^A | - | 0 | 1 | 1.2 | - | 0 | 1 | 1.4 |
| Pa ^B Pa ^B /Tf ^A Tf ^A /Am ^A Am ^B | 1 | 1.4 | - | 0 | 2 | 3 | 3 | 4.2 |
| Pa ^B Pa ^B /Tf ^A Tf ^A /Am ^B Am ^B | 2 | 2.6 | 2 | 2.7 | 4 | 6.1 | - | 0 |
| Pa ^B Pa ^B /Tf ^A Tf ^B /Am ^A Am ^A | - | 0 | 2 | 2.7 | - | 0 | - | 0 |
| Pa ^B Pa ^B /Tf ^A Tf ^B /Am ^A Am ^B | 6 | 7.9 | 7 | 9.2 | - | 0 | 3 | 4.2 |
| Pa ^B Pa ^B /Tf ^A Tf ^B /Am ^B Am ^B | 7 | 9.2 | 7 | 9.2 | 11 | 16.7 | 4 | 5.5 |
| Pa ^B Pa ^B /Tf ^B Tf ^B /Am ^A Am ^A | - | 0 | 2 | 2.6 | - | 0 | 1 | 1.4 |
| Pa ^B Pa ^B /Tf ^B Tf ^B /Am ^A Am ^B | 7 | 9.2 | 8 | 10.5 | - | 0 | 7 | 9.7 |
| Pa ^B Pa ^B /Tf ^B Tf ^B /Am ^B Am ^B | 30 | 39.5 | 18 | 23.7 | 15 | 22.7 | 23 | 31.9 |

A common point in the four samples studied is the high proportion of individuals with the Pa^BPa^B/Tf^BTf^B/Am^BAm^B genotypic combination.

The highest frequency for this genotypic combination category was determined in the Landrace sample, 16.8% higher than that determined in the LP 2000 sample.

From the data presented in Table 1 one can remark that there are differences among the genetic structures of the four samples, mainly by the absence of different categories with varied genotypic combinations in the respective samples. Thus, from the 27 possible genotypic combinations among the three loci, the Landrace breed population counts 13, and the Great White breed population 16. The sample belonging to the Synthetic Line P 2000 is distinguished by the smallest number of genotypic combination categories, respectively 12. Only 15 categories of genotypic combinations are found in the sample within the Synthetic Line LS 345. Also, the frequency of the genotype categories found in each of the four samples was different (Table 1).

One doesn't find individuals with the genotypic combinations $IPa^A Pa^A / Tf^A Tf^A / Am^A Am^A$ and $Pa^A Pa^A / Tf^A Tf^A / Am^A Am^B$ in any of the samples, as a consequence of the small share that individuals with type A of transferin had in each of the studied samples.

Equilibrium state estimation

A. Landrace sample

The genetic structure setting up allowed the estimation of the equilibrium state for the Landrace sample.

The frequencies of the twenty-seven categories of possible genotypic combinations among the three studied loci were organized in a matrix with the following structure:

$$G = \begin{bmatrix} 0 & 0 & 0 & 0 & 0.026 & 0 & 0 & 0 & 0.039 \\ 0 & 0 & 0 & 0 & 0.026 & 0.040 & 0.040 & 0.013 & 0.118 \\ 0 & 0.014 & 0.026 & 0 & 0.079 & 0.092 & 0 & 0.092 & 0.395 \end{bmatrix}$$

Knowing the frequencies of the genotypes categories, the population's gamete background was also determined. The frequencies of the

gamete categories were structured as a matrix, as it follows:

$$g = \begin{bmatrix} 0.010 & 0.020 & 0.033 & 0.121 \\ 0.030 & 0.112 & 0.092 & 0.582 \end{bmatrix}$$

In the case we studied, it wasn't found any genetic equilibrium revealed by the relationship:

$$\begin{aligned} &g_{11} \times g_{33} \times g_{17} \times g_{39} \neq g_{31} \times g_{13} \times g_{37} \times g_{19} \\ &\text{respectively:} \\ &0.010 \times 0.112 \times 0.033 \times 0.582 \neq 0.030 \times 0.020 \times 0.092 \times 0.121 \\ &0.0000215 \neq 0.0000067 \end{aligned}$$

B. Great White sample

The matrix structure according to the frequencies of the 27 aggregate genotypic

combinations which can result from the three loci is:

$$G = \begin{bmatrix} 0 & 0 & 0 & 0 & 0.026 & 0 & 0 & 0 & 0 \\ 0 & 0.053 & 0.013 & 0 & 0.040 & 0.039 & 0.013 & 0.066 & 0.132 \\ 0.012 & 0 & 0.027 & 0.027 & 0.092 & 0.092 & 0.026 & 0.105 & 0.237 \end{bmatrix}$$

The values of the gamete categories were placed in the form of a matrix, respectively:

$$g = \begin{bmatrix} 0.025 & 0.041 & 0.034 & 0.104 \\ 0.067 & 0.103 & 0.143 & 0.456 \end{bmatrix}$$

Having all the necessary data, there were compared the product values of the gametes categories frequency among which there must

be equality, under genetic equilibrium conditions:

$$g_{11} \times g_{33} \times g_{17} \times g_{39} = g_{31} \times g_{13} \times g_{37} \times g_{19}$$

$$0.025 \times 0.103 \times 0.034 \times 0.456 \neq 0.067 \times 0.041 \times 0.143 \times 0.104$$

$$0.000039 \neq 0.000041$$

Since the conditions of equilibrium are not satisfied, the Great White breed sample cannot be considered in genetic equilibrium at the moment of the study.

The Hardy-Weinberg equilibrium rarely applies in reality (www.nature.com).

C. P 2000 Synthetic Line Sample

As regards the state of equilibrium in the three loci, the genotypic matrix was established on the basis of genotypic combinations categories weights in the LP sample:

$$G = \begin{bmatrix} 0 & 0 & 0.015 & 0 & 0 & 0.061 & 0 & 0 & 0.121 \\ 0 & 0.015 & 0 & 0 & 0.061 & 0 & 0.076 & 0.136 & 0.030 \\ 0 & 0.030 & 0.061 & 0 & 0 & 0.167 & 0 & 0 & 0.227 \end{bmatrix}$$

For the gamete matrix, there was established the following relationship:

$$g = \begin{bmatrix} 0.011 & 0.057 & 0.080 & 0.208 \\ 0.026 & 0.171 & 0.080 & 0.367 \end{bmatrix}$$

In the case we studied, the equilibrium condition:

$$g_{11} \times g_{33} \times g_{17} \times g_{39} = g_{31} \times g_{13} \times g_{37} \times g_{19}$$

isn't fulfilled, respectively:

$$0.011 \times 0.171 \times 0.080 \times 0.367 \neq 0.026 \times 0.057 \times 0.080 \times 0.208$$

$$0.000055 \neq 0.000025$$

This means that the sample analysed did not have an equilibrium genetic structure. The Hardy-Weinberg equilibrium describes an idealized state, and genetic variations in nature can be measured as changes from this equilibrium state (www.nature.com).

D. 345 Synthetic Line Sample

In order to estimate the equilibrium state, according to the twenty-seven possible genotypic combinations among prealbumins, transferins and serum amylases, the genotypic matrix was drawn up:

$$G = \begin{bmatrix} 0 & 0 & 0 & 0 & 0.014 & 0.028 & 0 & 0.014 & 0.069 \\ 0 & 0 & 0 & 0 & 0 & 0.083 & 0.014 & 0.028 & 0.167 \\ 0.014 & 0.042 & 0 & 0 & 0.042 & 0.055 & 0.014 & 0.097 & 0.319 \end{bmatrix}$$

The gametic matrix was structured as follows:

$$g = \begin{bmatrix} 0.004 & 0.038 & 0.024 & 0.205 \\ 0.045 & 0.080 & 0.087 & 0.517 \end{bmatrix}$$

The frequencies of the gamete categories were determined according to the frequencies of the corresponding genotypic combination

categories. Knowing that the equilibrium state is achieved when the gametic matrix achieves equality:

$$g_{11} \times g_{33} \times g_{17} \times g_{39} = g_{31} \times g_{13} \times g_{37} \times g_{19}$$

it can be concluded that at the time of the study the sample within LS 345 did not have a genetic equilibrium structure, since:

$$0.004 \times 0.080 \times 0.024 \times 0.517 \neq 0.045 \times 0.038 \times 0.087 \times 0.205$$

$$0.000004 \neq 0.000030$$

CONCLUSIONS

The sample belonging to the Great White breed contains the most genotypic categories (16), and in the LP sample the fewest (12). The LS sample showed 15 genotypic categories, and the Landrace sample 13 categories. The frequency of the genotype categories found in each of the four samples was different.

Common to all four studied samples is the high proportion of individuals with the $Pa^B Pa^B / Tf^B Tf^B / Am^B Am^B$ genotypic combination.

The highest frequency for this genotypic combination category was determined in the Landrace breed sample (39.5%).

None of the four samples contain individuals with the following genotypic combinations $Pa^A Pa^A / Tf^A Tf^A / Am^A Am^A$ and $Pa^A Pa^A / Tf^A Tf^A / Am^A Am^B$, as a consequence of the small share that individuals with transferrin type A had in each of the studied samples.

No genetic balance for the PaTfAm aggregate genotype was identified in any investigated population. One has not identified a genetic equilibrium for the PaTfAm aggregate genotype in any of the investigated population

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