UDC 577.21; 636,082

EVALUATION OF THE FEASIBILITY OF MARKER ASSISTED SELECTION BY USING THE INDEXES OF POPULATION-GENETIC VARIABILITY

A. Saienko, Junior Researcher

V. Balatskyi, Ph.D.

Institute of Pig Breeding and Agro-Industrial production of the National Academy of Agricultural Sciences of Ukraine, Poltava

On the base of the calculated indexes of population-genetic variability: level of heterozygosity and fixation index of Wright it was evaluated the feasibility of selection on genetic markers of loci GH, PRLR, RYRI, ESR1 and IGF2 in breeds and types of pigs of the different productive direction. Marker assisted selection is possible in populations of the Ukrainian Large White breeds of line 1and line 3, also in populations of English Large White, Pietrain and Meishan on loci GH, PRLR, ESR1, IGF2 and in Large Black on GH, PRLR, ESR1. Marker assisted selection on locus RYRI cannot be performed because in all tested breeds it was monomorphic. In the Large Black breed locus IGF2 was monomorphic, and Meishan - GH, which also makes it impossible to carry out the marker assisted selection in them on these loci.

Population genetic variability, pig breed, heterozygosity, gene loci, polymorphism.

The process of selection in pig breeding can be more effective if for the evaluation of genotypes of animals and their subsequent selection in addition to traditional approaches researchers use molecular genetic marking animals on quantitative traits loci (QTL - quantitative trait loci). The latter ones involves a number of genes for which it has been proved the significant associations with specific reproductive, fattening and meat qualities of pigs. Taking into account the results of typing on such genes it is created the different lines of breeds, for example, with a high multiple pregnancy sows [6], or improving the meat conditions of existing breeds of pigs [3].

Population studies must precede the introduction of selection for QTL marker genes, and an integral part of such research is analysis of the primary data using statistical programs: GenAlEx 6, STATISTICA, Mega 4 et al., [9-11]. The calculation of a number of population parameters, especially the parameters of population genetic variability, which are based on different combinations of alleles [12], makes it possible to carry out a genetic analysis and monitoring of populations, and to determine the possibility of the marker assisted selection on selected QTL. A measure of a population genetic variability is primarily a level of heterozygosity. However, it should be noted that the observed population heterozygosity varies depending on many factors and especially on the degree panmixia. If the population consists of mainly of homozygotes, then the observed heterozygosity in general can not serve as an indicator of population genetic variability. Given these circumstances, it can determine the expected heterozygosity for a single locus, which could be in populations subject to a full panmixia [5]. So, it is the definition of expected heterozygosity can further serve as a basis for planning, both traditional and marker selection for QTL-genes in the studied populations in the future.

Specialized breeds or interbreed types are characterized by predominant expression of some productive traits. The Large White (LW) breed, which belongs to the breeds of an universal type of productivity, in Ukraine is represented by several lines, including Ukrainian Large White line 1 (ULW1) is a maternal type with improved reproductive qualities and Ukrainian Large White line 3 (ULW3) - with improved meat qualities, so-called paternal type. Also, there is a population of English Large White (LWE) of the meat productivity. The Large Black breed (LB) in Ukraine belongs to genotypes of fatty and meat type of productivity. Pietrain is the high meat productivity breed and Meishan is the fatty productivity breed. The analysis in these breeds and types of population genetic variability in relation loci *GH, IGF2, RYRI, PRLR,* and *ESR1,* of which the first three loci are associated mainly with meat and fattening qualities, and the rest with the reproductive traits may be interesting from the point of view of the feasibility of marker assisted selection in them, as in the direction of "specialization" of breeds and other traits to improve productivity.

The aim of the study was to assess the feasibility of marker assisted selection for some QTL genes in populations of pigs of different productivity based on indexes of the population genetic variability.

Materials and methods of researches. Biomaterials (blood) were taken from the ear vein of animals ULW1 and ULW3, LWE, LB from pedigree farms of

Ukraine. DNA samples of Meishan and Pietrain breeds were used from of DNA bank of Genetics Laboratory of Institute of Pig Breeding and AIP NAAS.

DNA was extracted by salt method [4] and DNA samples were stored at -20°C.

Polymerase chain reaction (PCR) was performed on PCR cycler machine "Tertsyk -2 '(DNA Technology, Russia). Restriction analysis of loci: insulin-like growth factor 2 (*IGF2*, insulin-like growth factor 2), ryanodine receptor (RYRI-ryanodine receptor gene), prolactin receptor gene (*PRLR*- prolactin receptor gene), growth hormone (*GH* - growth hormone gene) and estrogen receptor 1 (*ESR1* - oestrogen receptor gene 1) were performed according to the recommendations [1, 7, 14, 16, 17]. The mixture of restriction fragments mixture was analyzed by electrophoresis in 8% polyacrylamide gels. Sizes of received restriction DNA fragments were determined using molecular weight marker pBR322 / BsuRI. Statistical data processing was performed using standard software for population studies GenAlEx6.

Results. It was carried out the DNA typing studied pigs for gene loci *GH, PRLR, RYRI, ESR1* and *IGF2*. The results were used to determine observed and expected heterozygosity, and fixation index of Wright in Table 1, as indexes of population-genetic variability.

For a number of loci and populations it was revealed significant differences between observed and expected levels of heterozygosity. For example, for *GH* and *PRLR* loci in almost all the studied breeds the advantage of indexes He over Ho was observed, heterozygous genotype was much less than expected from their normal distribution calculated using GenAlEx 6. The deficit of heterozygotes for these loci may be associated with a predominantly unconscious artificial or natural selection of homozygous genotypes. Such shifts in the distribution of genotypes for locus *PRLR*, for example, was also observed in the population for the Polish Landrace and the Large White breeds [13], in Vietnamese pigs [15], Ukrainian and Welsh beef breeds [2], but it was not found in some others [8]. The statistically confirmed deficit of heterozygotes was observed also for *ESR1* locus in a population ULW3. In the above cases, the observed heterozygosity Ho does not display correctly allelic polymorphism of locus in a population where both alleles are present, but mainly in the homozygous state and, consequently, the observed heterozygosity is very low, or even equal to 0 (eg, locus *PRLR* in micropopulation of pigs Meishan and *GH* in the breed Pietrain). Therefore, to assess the feasibility of marker assisted selection appropriate to use He, which reflects the potential of a population on the possible combination of alleles in the next generation and holding of marker assisted selection. The coefficient of Wright is positive and shows a degree of inbreeding in this population, depending on the size of the coefficient. On the contrary, when the observed heterozygosity is higher than expected one in the population there is an excess of heterozygotes, which may be the result of selective pressure, or natural selection. In our opinion, in this situation the index He more correctly reflects the level of genetic variation that can potentially occur with a combination of alleles depend on their frequency in the following generation, if factors of selection will be not influence. Accordingly, Wright's coefficient is negative.

In the investigated populations the genetic variability with regard *RYR1* locus is absent and marker assisted selection on the interbreed basis is impossible. Locus ESR1 has rather high, both observed and expected heterozygosity in almost all breeds and types and the marker assisred selection has perspectives from the point of view of a sufficient level of genetic variability. The genetic variability for PRLR locus when it is measured at the level of expected heterozygosity, is quite high in all populations, but in these generations, basically, does not appear sufficiently for an efficient selection of animals of all possible genotypes. This situation is typical for the locus of *GH*. Regarding the locus *IGF2*, the genetic variability of breeds is different and basically, doesn't observed major disparities in Ho and He.

Thus, perspective breeds and types for marker selection for *ESR1* locus is ULW1, ULW3, LWE, LB, Pietrain and Meishan on loci *PRLR* and *GH* - ULW1, ULW3, LWE and LB on locus *IGF2* - ULW1, ULW3, LWE and Meishan.

Table

Observed and theoretically expected heterozygosity in different populations

and the value of the fixation index of Wright

Breeds	**Loci**	**Ho**	**He**	**F**
ULW-1 (104 heads of pigs)	*RYRI*	0,000	0,000	-
	ESR1	0,510	0,498	-0,024
	PRLR	0,125***	0,466	0,732
	GH	0,029***	0,305	0,905
	IGF2	0,067	0,065	-0,035
LB (100 heads of	*RYRI*	0,000	0,000	-

pigs)	*ESR1*	0,230	0,276	0,165
	PRLR	0,180***	0,449	0,599
	GH	0,030***	0,302	0,901
	IGF2	0,000	0,000	-
LWE (100 heads of pigs)	*RYRI*	0,000	0,000	-
	ESR1	0,620	0,493	-0,258
	PRLR	0,020***	0,241	0,917
	GH	0,060***	0,241	0,751
	IGF2	0,560	0,461	-0,215
ULW-3 (100 heads of pigs)	*RYRI*	0,000	0,000	-
	ESR1	0,230**	0,424	0,457
	PRLR	0,190***	0,474	0,599
	GH	0,180***	0,403	0,554
	IGF2	0,340	0,332	-0,025
Pietrain (9 heads of pigs)	*RYRI*	0,000	0,000	-
	ESR1	0,111	0,278	0,600
	PRLR	0,000	0,198	1,000
	GH	0,000*	0,346	1,000
	IGF2	0,000	0,198	1,000
Meishan (5 heads of pigs)	*RYRI*	0,000	0,000	-
	ESR1	0,800	0,480	-0,667
	PRLR	0,000*	0,480	1,000
	GH	0,000	0,000	-
	IGF2	1,000*	0,500	-1,000

H_o - observed heterozygosity

H_e - expected heterozygosity

F - fixation index of Wright

* p≥0,05, ** p≥0,01, *** p≥0,001 - thresholds for reliability the criterion of Fisher.

Conclusions and perspectives for further research.

To assess the possibility of marker assisted selection it should be used index He, which reflects the potential of a population on the possible combination of alleles in the next generations and performing marker selection.

Perspective breeds and types for performing marker assisted selection for ESR1 locus is ULW1, ULW3, LWE, LB, Pietrain and Meishan by loci PRLR and GH - ULW1, ULW3, LWE and LB by locus IGF2 - ULW1, ULW3, LWE and Meishan.

The marker assisted selection for locus RYRI is impossible, because in all studied breeds it was monomorphic (in ULW-1, LB, LWE, ULW-3 and Meishan only genotypes NN in Pietrain – nn). In LB breed locus IGF2 was monomorphic, and in Meishan - GH, which also makes it impossible to conduct the marker assisted selection in them on these loci.

List of references

1. Брэм Г. Использование в селекции свиней молекулярной генной диагностики злокачественного гипертемического синдрома (MHS) / Г. Брэм, Б. Бренинг // Генетика. - 1993. – Т. 29, №6. – С. 1009-1013.
2. Драгулян М.В. Генетичний поліморфізм свиней (SUS SCROFA DOMESTICA) української м’ясної та уельської порід за цито- та молекулярно-генетичними маркерами: Дис. канд. біол. наук.- Київ, 2014.- 163 с.
3. Епишко О. А. Гены, детерминирующие воспроизводительную функцию свиноматок / О. А. Епишко // Весці нацыянальнай акадэміі навук Беларусі.- 2008. -№ 2.
4. Соколов Б.П. Выделение высокомолекулярной еукариотической ДНК с использованием ацетата калия / Б.П. Соколов, В.В.Джемелинский // Млекулярная генетика, микробиология и вирусология.- 1989.-№6.-С.45-46.
5. Тоцький В. М. Генетика: підручник, 3-тє вид., випр. та доп. / В. М. Тоцький // “Астропринт”. - 2008. – 712 с.

6. Шейко И. П. Селекция на Повышение многоплодия свиноматок / И.П.Шейко, Н.А Лобан., О.Я. Василюк, Д.С. Драбинович // Весці нацыянальнай акадэміі навук Беларусі.- 2006. - № 3.
7. Arkadiusz Terman, Marek Kmieæ Associations between the prolactin receptor gene polymorphism and reproductive traits of boars // J Appl Genet 47(2), 2006, pp. 139–141.
8. Barreras Serrano A., Herrera Haro J.G., Hori-Oshima S., Gutierrez Espinosa A., Ortega Cerrilla M.E., Perez Perez J., Lemus Flores C., Kinejara Espinosa A.L., Gonzalez Arangure A., Soto Avila J.G. Prolactin Receptor (PRLR) Gen Polymorphism and Associations with Reproductive Traits in Pigs // Journal of Animal and Veterinary Advances.- 2009.- №8 (3).- P. 469-475.
9. http://www.anu.edu.au/BoZo/GenAlEx/
10. http://www.statsoft.ru
11. http://www.megasoftware.net
12. http://vocabulary.ru/dictionary/873/word/geneticheskaja-izmenchivost
13. Kmiec M., Terman A. Associations between tbe prolactin receptor gene polymorphism and reproductive traits of boars // J. Appl. Genet. — 2006. — Vol. 47. — № 2. — P. 139–141.
14. Knoll A, Putnova L, Dvorak J, Cepia S. A *Nci* I *PCR-RFLP* within intron 2 of the porcine insulin-like growth favtor-2 (IGF2) gene J. Anim. Genet. 31 (2000) 150-151
15. Nguyen Thi Dieu Thuy Nguyen Thu Thuy and Nguyen Van Cuong. Genetic polymorphism of prolactin receptor gene in Mong Cai pig // Proceedings of International Workshop on Biotechnology in Agriculture. ABSTRACT MATERIALS AND METHODS. 2011-12-01. Source: asag-biotech.net http://www.findthatpdf.com/search-28520246-hPDF/download-documents-ntdthuy-pdf.htm.
16. Short H.T., Rothschild F. M. Effect of the Estrogen Receptor Locus on Reproduction and Production Traits in Four Commercial Pig Lines // J. Anim. Sci. 1997. 75:3138–3142.

17. Z. Vykoukalová, A. Knoll, J. Dvořák & Čepica New SNPs in the IGF2 gene and backfat thickness and lean meat content in Large White pigs. J. Anim. Breed. Genet. 123 (2006) 204-207