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MOLECULAR GENETIC ASSESSMENT OF RELATIONSHIPS AMONG THREE PIG BREEDS BASED ON *ESR1* AND *PRLR* GENES AND THEIR IMPORTANCE FOR SELECTION AND IMPLEMENTATION OF THE HETEROSIS EFFECT

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Objective. This study aimed to compare the Myrhorod, Poltava Meat, and Welsh pig breeds at the *ESR1* and *PRLR* loci, calculate genetic distances between breeds, and evaluate the practical implications of the findings for breeding programs and hybridization, with consideration of possible heterosis effect. **Methods**. DNA samples were collected from 20 individuals of each breed, maintained on farms in the Poltava region. Genomic DNA was extracted using Chelex 100. Genotyping of ESR1 and PRLR loci was performed via PCR-PCRF, including PCR amplification followed by analysis of restriction fragments on polyacrylamide gels. Allele and genotype frequencies were calculated, and observed and expected heterozygosity (Ho, He), polymorphic information content (PIC), and inbreeding coefficients (F) were determined. Genetic distances between breeds were estimated using Nei's algorithm, and breed relationships were visualized using the UPGMA method. **Results**. In the Myrhorod breed, allele A predominated for both *ESR1* (0.65) and *PRLR* (0.74), resulting in comparatively low genetic diversity (He = 0.38–0.45; PIC = 0.31–0.35). The Poltava Meat breed showed a more balanced allele distribution (*ESR1*: A = 0.58; *PRLR*: A = 0.51), with higher heterozygosity (Ho = 0.50–0.63) and locus informativeness (PIC = 0.37). In the Welsh breed, allele B was dominant (*ESR1*: 0.52; *PRLR*: 0.58), and the observed heterozygosity for *PRLR* (Ho = 0.762) was statistically higher than expected heterozygosity (He = 0.49), likely reflecting selection favoring heterozygotes. Genetic distance analysis revealed the greatest divergence between Myrhorod and Welsh breeds (0.125) and the smallest between Poltava Meat and Welsh breeds (0.019), with an intermediate distance observed between Myrhorod and Poltava Meat breeds (0.052), consistent with the historical contribution of Myrhorod pigs to the Poltava Meat breed. The UPGMA dendrogram confirmed two main clusters: Poltava Meat and Welsh breeds are located together on the dendrogram, while Myrhorod breed is located separately, reflecting both breed specialization and genetic relationships. **Conclusions**. The findings indicate that the genetically distant Myrhorod and Welsh breeds are suitable candidates for hybridization programs aimed at exploiting heterosis, while crosses between Poltava Meat and Welsh breeds may optimize meat productivity by consolidating desirable traits. The genetic proximity of Myrhorod and Poltava Meat breeds suggests their potential for maintaining local gene pools and enhancing population viability.

Keywords: genetic distance, marker-assisted selection, DNA markers, single nucleotide polymorphism, pig breeds, ESR1, PRLR.

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Introduction. Modern pig breeding increasingly combines classical breeding methods with advances in molecular genetics. The main goal of breeding programs remains the formation of livestock that surpasses previous generations in terms of a complex of productive and reproductive traits. The implementation of this task is based on the use of crossing and interbreed hybridization, which makes it possible to realize the effect of heterosis–an increase in the productivity, viability, and reproductive capacity of hybrids compared with parental forms.

The magnitude of the heterosis effect is largely determined by the level of genetic distance between the breeds involved. The more diverse their genetic pool, the higher the probability of obtaining offspring with optimal combinations of desirable alleles [1–4]. In contrast, excessive relatedness or the uncontrolled use of closely related lines contributes to the accumulation of inbreeding, which can lead to reduced viability, deterioration of reproductive performance, and the loss of economically valuable traits. In this regard, the assessment of genetic distances between breeds is an important component of scientifically grounded planning of breeding schemes.

Of particular importance is the analysis not only of the overall level of genetic variability but also of the variability of individual loci that are directly related to the productive and reproductive characteristics of pigs. In this context, the *ESR1* (estrogen receptor 1) and *PRLR* (prolactin receptor) genes are of considerable interest. Polymorphism of the ESR1 gene is associated with sow prolificacy, the duration of reproductive use, and overall reproductive efficiency [5–7]. The *PRLR* gene plays a key role in the regulation of lactation and mammary gland development and also indirectly affects offspring survival and growth [8–10].

The study of variability in the *ESR1* and *PRLR* genes makes it possible to assess genetic differences between breeds, identify promising combinations for interbreed crossing aimed at improving reproductive performance, cluster populations to determine their degree of relatedness and genetic uniqueness, and contribute to the preservation of local gene pools through the control of inbreeding and the maintenance of genetic diversity.

Thus, the use of the *ESR1* and *PRLR* genes as markers of quantitative traits expands the possibilities for developing effective breeding programs focused not only on improving meat productivity but also on optimizing the reproductive characteristics of pigs.

The aim of this work was to compare the Myrhorod, Poltava Meat, and Welsh pig breeds at the *ESR1* and *PRLR* loci, to calculate genetic distances between breeds, and to outline the practical significance of the obtained results for breeding practice and reproduction systems.

Materials and methods. The study was conducted using DNA samples obtained from pigs of three breeds: the Myrhorod breed (a local Ukrainian breed of a dual-purpose type, $n = 20$), the Poltava Meat breed (a domestic meat-type breed, $n = 20$), and the Welsh breed (a foreign breed characterized by high meat productivity, $n = 20$). The animals originated from private agricultural enterprise “ORACH” and farm “SAM-12” located in the Poltava region, Ukraine.

DNA extraction was performed using a rapid method with the Chelex 100 reagent [11]. The resulting DNA preparations were stored at –20 °C until further analysis. Polymerase chain reaction (PCR) was carried out using a “Tertsyk-2” thermal cycler. PCR amplification and restriction fragment length polymorphism

(PCR-RFLP) analysis [12, 13] of the *PRLR* and *ESR1* loci were performed in accordance with previously published protocols [14, 15]. The restriction fragments were separated by electrophoresis in an 8 % polyacrylamide gel. Fragment sizes were determined using the pBR322/MspI molecular weight marker. The PCR amplification parameters and PCR-RFLP patterns are presented in Table 1.

Table 1. Parameters of *PRLR* and *ESR1* loci analysis in PCR-RFLP

Gene	Structure of primers for PCR	Amplicon length	m	Restriction enzyme and fragments' sizes
ESR1	F: 5'-CCTGTTTTTACAGTGACTTTTACA GAG-3' R: 5'-CACTTCGAGGGTCAGTCCAATTAG-3'	120	6	*Pvu* II: A allele: 120 bp B allele: 65 bp, 55 bp
RPRL	F: 5'-CGTGGCTCCGTTTGAAGAACC-3' R: 5'-CTGAAAGGAGTGCATAAAGCC-3'	163	0	*Alu* I: A allele: 85 bp, 59 bp, 19 bp B allele: 104 bp, 59 bp

Statistical analysis of the data was performed using the GenAlEx 6.5 add-in [16] within the Microsoft Office Excel 2019 environment. Genetic distances between breeds were calculated using Nei's genetic distance algorithm [17]. To visualize genetic relationships among the studied breeds, a dendrogram was constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method [18] implemented in the MEGA4 software package [19].

The use of animals in this study was approved by the Scientific Council of the Institute of Pig Breeding and Agro-Industrial Production (protocol No.1 dated 08.01.2024) and was conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18 March 1986).

Results and Discussion. During the analysis of genotyping results across the three pig breeds, allele and genotype frequencies at the *ESR1* and *PRLR* loci were determined for the Myrhorod, Poltava Meat, and Welsh pig populations. The obtained results revealed differences in allele and genotype distributions among the populations, which was reflected in the levels of heterozygosity and the values of the polymorphism information content (PIC).

The population genetic characteristics of the studied breeds (Table 2) indicate breed-specific patterns of genetic variability at both loci.

In the Myrhorod breed, allele A predominated at both the *ESR1* (0.65) and *PRLR* (0.74) loci. This allelic distribution resulted in relatively lower levels of genetic diversity, with expected heterozygosity (He) equal to 0.38 for *PRLR* and to 0.454 for *ESR1*, while PIC values were 0.31 for *PRLR* and 0.35 for *ESR1*. These results indicate moderate marker informativeness and suggest a tendency toward the preservation of a traditional allelic structure within this local breed.

In the Poltava Meat breed, allele frequencies were more balanced. The frequency of A allele was 0.58 for *ESR1* and 0.51 for *PRLR* loci, which was associated with higher heterozygosity levels and PIC values reaching 0.37. This pattern may reflect a relatively broader genetic base and a greater potential for improving productive traits through the application of appropriate breeding schemes.

Table 2. Population genetics characteristics of studied pig breeds

Breed	Locus	Genotype frequencies			χ^2	F	Ho	He	PIC
		AA	AB	BB					
Myrhorod	*ESR1*	0.46 (0.43)	0.39 (0.45)	0.15 (0.12)	0.870	0.138	0.391	0.454	0.35
	PRLR	0.55 (0.55)	0.38 (0.38)	0.07 (0.07)	0.002	0.007	0.378	0.380	0.31
Poltava Meat	*ESR1*	0.27 (0.34)	0.63 (0.49)	0.10 (0.17)	2.752	–0.303	0.633	0.486	0.37
	PRLR	0.26 (0.27)	0.50 (0.50)	0.24 (0.24)	0.000	–0.001	0.500	0.500	0.37
Welsh	*ESR1*	0.19 (0.23)	0.57 (0.50)	0.24 (0.27)	0.444	–0.145	0.571	0.499	0.37
	PRLR	0.05 (0.18)	0.76 (0.49)	0.19 (0.33)	6.481*	–0.556	0.762	0.490	0.37

Note indicates the significant deviation of Ho from He under Hardy-Weinberg equilibrium*

In the Welsh breed, a predominance of allele B was observed for both genes: its frequency was 0.52 for *ESR1* and 0.58 for *PRLR* loci. At the same time, a high level of observed heterozygosity was detected, particularly for the *PRLR* gene (Ho = 0.762), which exceeded the expected heterozygosity (He = 0.490). Such a discrepancy may indicate the action of selection favoring the maintenance of heterozygous genotypes and suggest potential adaptive advantages associated with these genotypes in the population.

The obtained allele and genotype frequencies enabled the calculation of genetic distances between the studied breeds, which represent a key indicator for assessing the feasibility of crossbreeding strategies. According to the calculated values (Table 3), the maximum genetic distance was observed between the Myrhorod and Welsh breeds (0.125), indicating substantial differences in their genotypic structure. In contrast, the minimum genetic distance was detected between the Poltava Meat and Welsh breeds (0.019), submitting a high degree of genetic similarity and convergence in productive orientation. An intermediate genetic distance (0.052) was recorded between the Myrhorod and Poltava Meat breeds, reflecting their historical genetic relationship, as the Myrhorod breed contributed to the development of the Poltava Meat breed.

Table 3. Nei's genetic distances between the studied breeds based on *PRLR* and *ESR1* loci

№	**Breeds**	**1**	**2**	**3**
1	Myrhorod	****	0.052	0.125
2	Poltava Meat	0.052	****	0.019
3	Welsh	0.125	0.019	****

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The constructed dendrogram (Figure 1) revealed the formation of two major clusters.

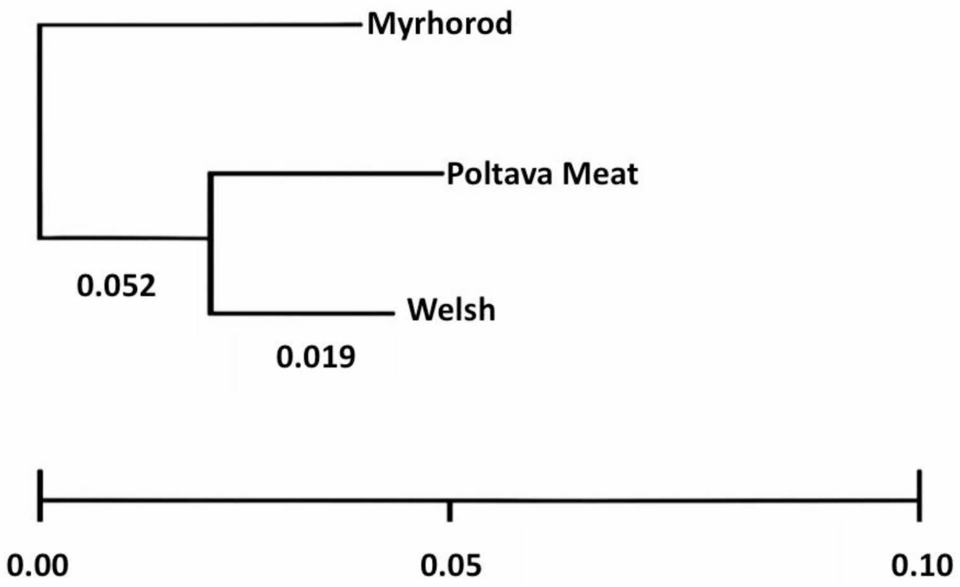

Figure 1. Dendrogram of genetic relationships among three pig breeds based on allele frequencies at ESR1 and PRLR loci, constructed using the UPGMA method

The Poltava Meat and Welsh breeds clustered together at the earliest stage due to their minimal genetic distance and shared meat-oriented productive direction. The Myrhorod breed joined the cluster at a subsequent stage, indicating its greater genetic distinctiveness while simultaneously confirming its historical contribution to the formation of the Poltava Meat breed.

The high level of genetic divergence between the Myrhorod and Welsh breeds (0.125) indicates the advisability of their use in hybridization programs aimed at achieving a heterosis effect. The pronounced genetic similarity between the Poltava Meat and Welsh breeds (0.019) suggests their effective joint use in meat-oriented breeding programs to enhance desirable productive traits. The moderate genetic proximity between the Myrhorod and Poltava Meat breeds (0.052) supports the feasibility of their combination for the conservation of genetic resources and the maintenance of local Ukrainian pig populations.

Overall, the results confirm pronounced interbreed differentiation and demonstrate the practical value of applying *ESR1* and *PRLR* markers in pig breeding. Comparison of the obtained data with previously published studies supports general trends reported in the literature, indicating that the *ESR1* and *PRLR* loci are informative markers for evaluating reproductive and productive traits in pigs [20-22]. Moreover, numerous authors emphasize that the maximum heterosis effect is achieved when crossing breeds that differ substantially in the allelic composition of key loci, which is consistent with the observed contrast between local (Myrhorod and Poltava Meat) and foreign (Welsh) pig populations in the present study.

Conclusions. It was established that the greatest genetic distance was observed between the Myrhorod and Welsh breeds (0.125), whereas the minimum genetic distance (0.019) was recorded between the Poltava Meat and

Welsh breeds. The constructed dendrogram confirmed the formation of two major clusters – Poltava Meat with Welsh and Myrhorod – which is consistent with the breeding history and productive specialization of these breeds. The obtained results are of practical significance for the development of hybridization strategies, the conservation, restoration of local pig populations, and the prevention of inbreeding.

Prospects for further research. Further studies should focus on expanding the set of quantitative trait genes included in the analysis, involving a broader range of local Ukrainian pig breeds to assess their genetic uniqueness, incorporating highly productive foreign breeds as genetic donors in programs aimed at improving meat quality, and developing integrated breeding schemes that combine traditional productivity evaluation with modern genomic approaches.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧНА ОЦІНКА ВЗАЄМОВІДНОСИН МІЖ ТРЬОМА ПОРОДАМИ СВИНЕЙ ЗА ГЕНАМИ *ESR1* ТА *PRLR* ТА ЇХ ЗНАЧЕННЯ ДЛЯ СЕЛЕКЦІЇ Й РЕАЛІЗАЦІЇ ЕФЕКТУ ГЕТЕРОЗИСУ

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Мета. *Метою цього дослідження було порівняння миргородської, полтавської м'ясної та уельської порід свиней за локусами ESR1 та PRLR, розрахунок генетичних відстаней між породами та оцінка практичного значення отриманих результатів для селекційних програм та гібридизації з урахуванням можливого ефекту гетерозису.* ***Методи.*** *Зразки ДНК були зібрані у 20 особин кожної породи, що утримуються на фермах Полтавської області. Геномну ДНК екстрагували за допомогою Chelex 100. Генотипування локусів ESR1 та PRLR проводили за допомогою ПЛР-ПДРФ, включаючи ПЛР-ампліфікацію з подальшим аналізом рестрикційних фрагментів на поліакриламідних гелях. Були розраховані частоти алелів та генотипів, а також визначені*

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фактична та очікувана гетерозиготність (Ho, He), вміст поліморфної інформації (PIC) та коефіцієнти інбридингу (F). Генетичні відстані між породами оцінювали за допомогою алгоритму Нея, а породні зв'язки візуалізували за допомогою методу UPGMA. ***Результати****. У миргородській породі алель А переважав як для ESR1 (0,65), так і для PRLR (0,74), що призвело до порівняно низької генетичної різноманітності (He = 0,38–0,45; PIC = 0,31-0,35). Полтавська м'ясна порода показала збалансованіший розподіл алелів (ESR1: A = 0,58; PRLR: A = 0,51) з вищою гетерозиготністю (Ho = 0,50–0,63) та інформативністю локусу (PIC = 0,37). В уельській породі алель В був домінантним (ESR1: 0,52; PRLR: 0,58), а спостережена гетерозиготність для PRLR (Ho = 0,762) була статистично вищою за очікувану гетерозиготність (He = 0.49), ймовірно, відображаючи відбір на користь гетерозигот. Аналіз генетичних дистанцій виявив найбільшу дивергенцію між миргородською та уельською породами (0,125) та найменшу – між полтавською м'ясною та уельською (0,019), при цьому проміжна дистанція спостерігалася між миргородською та полтавською м'ясною породами (0,052), що узгоджується з історичним внеском миргородських свиней у полтавську м'ясну породу. Дендрограма UPGMA підтвердила два основні кластери: полтавська м'ясна та уельська породи розміщуються разом на дендрограмі, тоді як миргородська порода розміщується окремо, що відображає як спеціалізацію породи, так і генетичні зв'язки.* ***Висновки.*** *Результати дослідження свідчать, що генетично віддалені миргородська та уельська породи є придатними кандидатами для програм гібридизації, спрямованих на використання гетерозису, тоді як схрещування полтавської м'ясної та уельської порід може оптимізувати м'ясну продуктивність шляхом консолідації бажаних ознак. Генетична близькість миргородської та полтавської м'ясної порід свідчить про їхній потенціал для підтримки локальних генофондів та підвищення життєздатності популяції.*

Ключові слова: *генетична дистанція, маркер-асистований відбір, ДНК-маркери, однонуклеотидний поліморфізм, породи свиней, ESR1, PRLR.*

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