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THE OESTROGEN RECEPTOR GENE (*ESR*) *PVU*II POLYMORPHISM GENOTYPE AND ALLELE FREQUENCIES IN CZECH LARGE WHITE AND LANDRACE

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Abstract

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Genotype and allele frequencies of the oestrogen receptor gene (*ESR*) *Pvu*II restriction site were investigated in populations of the main Czech maternal breeds. 1253 sows and gilts and 396 boars in Large White and 334 sows and gilts and 318 boars in Landrace were genotyped from blood samples by the modified PCR-RFLP procedure as described in Short et al. (1997). In Large White, the frequency of allele *B* was about 0.51. In Landrace, the frequency of allele *B* reached from 0.02 for boars to 0.03 for sows. No significant deviations of the observed genotype frequencies from the frequencies expected according to Hardy-Weinberg equilibrium were found in both breed. Opposite trends in allele frequencies development could be assumed for analysed sows and boars of both breeds. In Large White sows the frequency of allele *B* raised probably due prefering sows with *BB* genotype, whereas in Landrace population the frequency of allele *B* decreased from yet unknown reasons.

pig, litter size, candidate gene, *ESR*, allele frequencies

Efficiency of livestock production is highly influenced by reproductive success (Short et al., 1997). The genetic progress obtained in large national purebred populations is often much less than supposed (van der Lende et al., 2002) due to the nature of reproduction traits (low heritability, sex-limited, performance late in life etc.). Information about the identification of individual genes or anonymous genetic markers associated with litter size could contribute to an increased rate of genetic gain achieved in pig populations.

Association between polymorphisms at several candidate genes and reproductive performance have been reported (Kirkpatrick, 2002). The *Pvu*II polymorphism of the oestrogen receptor (*ESR*) gene has been shown to influence sow prolificacy in USA and European Meishan synthetics (Rothschild et al., 1995; Southwood et al., 1995). These results were confirmed for Large White populations as well (Rothschild et al., 1995; Short et al., 1997). Estimates of the *ESR* polymorphism effect in the first parities varied from 1.43 piglets per litter in Meishan synthetics to 0.42 piglets per litter in Large White lines, both per copy of allele *B*. In other studies no clear association between the *ESR* polymorphism and litter size traits was shown (Southwood et al., 1995; Rothschild et al., 1996; Legault et al., 1996). *Pvu*II polymorphism of *ESR* gene was detected in the Czech Republic in

Large White and Landrace populations (Kolaříková et al., 2000; Putnová et al., 2001; Vrtková and Dvořák, 2001) and in Prestice Black Spotted population as well (Nováková, 2003).

The aim of the present paper was to investigate *ESR*-*Pvu*II genotype and allele frequencies and their development in both main Czech maternal pig breed populations.

MATERIAL AND METHODS

Genotype and allele frequencies of the oestrogen receptor gene (*ESR*) *Pvu*II restriction site were investigated for a data set including 1253 gilts and sows and 396 boars of Large White and 334 gilts and sows and 318 boars of Landrace breed. Large White gilts and sows (next sows) originated from 34 nucleus herds and Landrace sows from 8 nucleus herds. Sow's genotype data gathering was connected with the creation of Large White and Landrace hyperprolific lines. All animals were genotyped for the porcine oestrogen receptor gene (*ESR*). The *Pvu*II polymorphism was analysed by the PCR–RFLP procedure from blood samples. The PCR with primer sequences as described in Short et al. (1997) was used to amplify DNA samples. The 120 bp long PCR product was in the presence of allele *B* cut into fragments of length 55 bp and 65 bp, whereas in the presence of allele *A* the PCR product remained intact. In the Czech Republic were for allele marking used letters *C* and *D*, in the present study are used letters according to original method: *A* and *B* for *C* and *D*, respectively. Allele and genotype frequencies were calculated within each breed. The Chi-square test was used to determine the presence of Hardy-Weinberg equilibrium for the *ESR* locus in each population.

RESULTS AND DISCUSSION

Genotype and allele frequencies in both main Czech maternal pig populations are very different (Tab. I and II). Whereas for Large White the frequency of the *B* allele reached values of about 0.51, in the Landrace population were observed frequencies of 0.02 for boars and 0.03 for sows. In contrast to the presence of all genotypes in Large White, in Landrace only *AA* and *AB* genotyped animals were found. Both Large White and Landrace populations were in Hardy-Weinberg equilibrium for the analysed locus.

These results are in accordance with findings in other populations in the world. Whereas the frequency of allele *B* ranged from 0.38 to 0.72 in Large White or Large White based populations (Rothschild et al., 1995; Southwood et al., 1995; Short et al., 1997; Leeds et al., 2002; Vrtková and Dvořák, 2001), in Landrace based populations allele *B* disappeared or its frequency is very law (Tab. III). Although values of allele *B* frequencies were in various Landrace populations similar, sows with *BB* genotype were observed only in two studies (Noguera et al., 2003; Vrtková and Dvořák, 2001). The absence of sows with *BB* genotype in some studies could be explained by the low freqency of allele *B* together with small number of genotyped animals. But further possible explanation (the impact of selection pressure on traits in breeding objective, relation between *BB* genotype and fitness) cannot be clearly excluded.

Trends in the development of allele frequencies in both investigated maternal breeds are presented in Tab. I. and II. Whereas for Large White boars the allele frequencies between years of birth are stable, for sows the frequency of allele *B* raises. The frequency of allele *B* for sows born before 1999 achieved 0.37. This value is in accordance with outcomes found by Vrtková and Dvořák (2001). The frequency of allele *B* in following years raises and in sows born in 2002 achieved almost 0.58. The increasing of the allele *B* frequency in Large White sows was probably made by preferring sows with genotype *BB*.

Although only small number of *AB* genotyped animals was observed, the opposite trend in allele frequency development could be assumed in Landrace breed. This trend is confirmed by comparisson of the overall allele *B* frequency 0.03 with higher frequency 0.09 found in the year 2001 by Vrtková and Dvořák (2001). Rothschild et al. (1996) suggested that the *ESR*-*Pvu*II polymorphism is mainly restricted to commercial lines with Large White due to interbreeding of Chinese pigs with Large White ancestors in England prior to the 1800s. Noguera et al. (2003) assumed that similarly as in Large White populations, the allele *B* found in Spanish Landrace originated from Chinese breeds. *ESR*-*Pvu*II polymorphism was found in the most of analysed Landrace populations. Only sows of German Landrace breed were for this restriction site *AA* monomorphic. Then consider the number of genotyped sows, the absence of *ESR*-*Pvu*II polymorphism was not probably made by error in way of data gathering. From study of Steinheuer et al. (2002) could be deduced, that analysed polymorphism in German Landrace exist (at least in past). These authors did not find any polymorphism for sows involved in the study, but small fraction of analysed litters descended from boars with genotype *AB*.

In selected populations, a deviation from the Hardy-Weinberg equilibrium should be expected for genes, which have significant impact on the traits under selection. Although in Large White data set the performance of *AA* genotyped sows was about 0.5 piglets per litter greater ($P < 0.01$) than the litter size of *BB* genotyped sows (Goliášová and Wolf, 2004), any deviation from Hardy-Weinberg equilibrium was not detected. Similarly to our result, in INRA hyperprolific and control lines no significant deviation

I: *Development of ESR-PvuII genotype and allele frequencies in Large White females and boars*

Year of birth	N	Genotype frequencies (%)			Allele frequencies		χ^2
		AA	*AB*	*BB*	*A*	*B*	
Sows and gilts							
1995-1998	150	40.00	46.67	13.33	0.633	0.367	0.003
1999	166	24.10	50.00	25.90	0.491	0.509	0.000
2000	420	22.14	50.48	27.38	0.474	0.526	0.064
2001	396	21.21	51.01	27.78	0.467	0.533	0.240
2002	121	24.79	33.06	42.15	0.413	0.587	12.260
All	1253	24.50	48.44	27.06	0.487	0.513	1.165
Boars							
1994 – 1999	73	23.29	53.42	23.29	0.500	0.500	0.342
2000	144	20.83	56.94	22.22	0.493	0.507	2.787
2001	150	25.33	46.67	28.00	0.487	0.513	0.653
2002	29	27.59	44.83	27.59	0.500	0.500	0.310
All	396	23.48	51.77	24.75	0.494	0.506	0.499

χ^2 – value of Chi-square test
Bold letters: Observed and expected genotype frequencies differ with $P < 0.01$

II: *Development of ESR-PvuII genotype and allele frequencies in Landrace females and boars*

Year of birth	n	Genotype frequencies (%)			Allele frequencies		χ^2
		AA	*AB*	*BB*	*A*	*B*	
Sows and gilts							
1996 - 1999	56	85.71	14.29	0	0.929	0.071	0.331
2000	103	90.29	9.71	0	0.951	0.049	0.268
2001	107	97.20	2.80	0	0.986	0.014	0.022
2002	68	100.00	0.00	0	1.000	0.000	-
All	334	93.71	6.29	0	0.969	0.031	0.351
Boars							
1996 - 1998	29	96.55	3.45	0	0.983	0.017	0.009
1999	45	88.89	11.11	0	0.944	0.056	0.156
2000	77	97.40	2.60	0	0.987	0.013	0.013
2001	106	94.34	5.66	0	0.972	0.028	0.090
2002	61	100.00	0.00	0	1.000	0.000	-
All	318	95.60	4.40	0	0.978	0.022	0.161

See Tab. I for the definition of symbols

III: *Frequency of allele B in ESR-PvuII restriction site in various Landrace pig populations*

Country	Number of sows	Allele *B* freq.	Author
Germany	709	0	Drőgeműller et al. (1999)
Germany	1672	0	Drőgeműller et al. (2001)
Germany	214	0	Drőgeműller et al. (2001)
Czech Rep.	157	0.03 to 0.10	Putnová et al. (2001)
Czech Rep.	284	0.09	Vrtková and Dvořák (2001)
Germany	125	0	Steinheuer et al. (2002)
Poland	207	0.06	Kmiec et al. (2002)
Poland	195	0.03 to 0.10	Natoloczna-Kotara (2002)
Slovakia	132	0.08	Omelka (2003)
Spain	288	0.10	Noguera et al. (2003)

from the Hardy-Weinberg equilibrium was observed. Although the lines significantly differed in litter size, the frequency of the *B* allele was similar in both lines (Legault et al., 1996). Linville et al. (2001) found no influence of selection on the *ESR* genotype frequencies as well. It should be taken into account that in both experiments only limited samples of animals were involved.

The existence of pleiotropic effects may explain why the different alleles at a locus associated with litter size remain in the population, even when under selection (van Rens, 2001). But until recently it is not obvious, whether the estimated allelic effects are caused by the *ESR* polymorphism directly or via an unknown linked major gene for litter size. The influence of the *ESR* gene on litter size differs between populations in size and also in allele of favour. *ESR* is not the sole gene influencing reproduction traits and so differences between populations could be explained by differences in allelic frequencies of other genes in the genetic background.

SOUHRN

Frekvence genotypů a alel *Pvu*II polymorfismu genu estrogenového receptoru (*ESR*) u plemene bílé ušlechtilé a landrase

U populací hlavních mateřských plemen chovaných v České republice byly zjišťovány frekvence genotypů a alel *Pvu*II restrikčního místa genu pro estrogenový receptor (*ESR*). Ze vzorků krve 1253 plemenic a 396 kanců plemene bílé ušlechtilé a 334 plemenic a 318 kanců plemene landrase byly určeny genotypy *ESR* modifikovanou metodikou PCR-RFLP podle Shorta et al. (1997). U kanců i prasnic plemene bílé ušlechtilé byla zjištěna frekvence alely *B* 0,51. U plemene landrase byla frekvence alely *B* 0,02 u kanců a 0,03 u prasnic. Genotypové frekvence u obou plemen nevykazovaly průkaznou odchylku od očekávaných frekvencí podle Hardy-Weinberga. Vývoj frekvencí alel poukazuje u plemene bílé ušlechtilé na zvyšující se frekvenci alely *B*, pravděpodobně způsobenou záměrnou selekcí zvířat genotypu *BB*. U plemene landrase byl pozorován opačný trend, který nemůže být na současné úrovni zjištění jednoznačně vysvětlen.

prase, velikost vrhu, kandidátní geny, *ESR*, frekvence

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REFERENCES

DRŐGEMŰLLER, C., HAMANN, H., DISTL, O.: Candidate gene markers for litter size in different German pig lines. Journal of Animal Science, 2001, 79, 2565-70.

DRŐGEMŰLLER, C., HAMANN, H., THIEVEN, U., KRIETER, J., DISTL, O., HARLIZIUS, B.: Influence of the genome region surrounding the estrogen receptor (*ESR*) gene on litter size in a German Landrace population. Archiv für Tierzucht, 1999, 42, 175-7.

GOLIÁŠOVÁ, E., WOLF, J.: Impact of the *ESR* gene on litter size and production traits in Czech Large White pigs. Animals Genetics, 2004, 35 (4), 293-7.

KIRKPATRICK, B. W.: QTL and candidate gene effects on reproduction in livestock: progress and prospects. In: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production (CD-ROM), Montpellier, 2002, Communication 08-01.

KMIEC, M., DVOŘÁK, J., VRTKOVÁ, I.: Study on a relation between estrogen receptor (*ESR*) gene polymorphism and some pig reproduction performance characters in Polish Landrace breed. Czech J. Anim. Sci., 2002, 47, 189-193.

KOLAŘÍKOVÁ, O., PUTNOVÁ, L., VRTKOVÁ, I., DVOŘÁK, J.: Genetics and molecular markers of porcine reproduction. In: Sb. XVIIth The international Conference on Reproduction of Farm Animals, 1.–2. 6. 2000, Liptovský Ján, 149-153 pp.

LEEDS, T. D., IRVIN, K. M., MOELLER, S. J.: The association between the estrogen receptor locus and growth, carcass and developmental traits in pigs. In: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production (CD-ROM), Montpellier, 2002, Communication 03-26.

LEGAULT, C., GRUAND, J., LEBOST, J., GARREAU, H., OLLIVIER, L., MESSER, L. A., ROTHSCHILD, M. F.: Fréquence et effet sur la prolificité du gčne *ESR* dans deux lignées Large White en France. Journées de la Recherche Porcine en France, 1996, 28, 9-14.

LINVILLE, R. C., POMP, D., JOHNSON, R. K., ROTHSCHILD, M. F.: Candidate gene analysis for loci affecting litter size and ovulation rate in swine. Journal of Animal Science, 2001, 79, 60-7.

NATOŁOCZNA-KOTARA, A.: Polimorfizm fragmentów restrykcyjnych w locus receptora estrogenowego (*ESR*) świń użytkowanych w Polsce i jego związek z Cechami reprodukcyjnymi oraz tucznymi i rzeźnymi loch rasy PBZ i WBP, The Agricultural University of Wrocław The Faculty of Biology and Animal Science, 2002, 135 pp.

NOGUERA, J. L., VARONA, L., GÓMEZ-RAYA, L., SÁNCHEZ, A., BABOT, D., ESTANY, J., MESSER, L. A., ROTHSCHILD, M., PÉREZ-ENCISO, M.: Estrogen receptor polymorphism in Landrace pigs and its association with litter size performance. Livestock Production Science, 2003, 82, 53-9.

NOVÁKOVÁ, J.: [Crossbreeding of pigs with using of Prestice Black Pied (Pc)], Hybridizace prasat s využitím přeštického černostrakatého plemene. Ph.D. thesis, University of South Bohemia České Budějovice Faculty of Agriculture, 2003, 98 pp.

OMELKA, R.: [Analysis of some genetic polymorphisms in relationship to reproduction traits in pigs], Analýza vybraných genetických polymorfizmov vo vzťahu k reprodukčným vlastnostiam ošípaných, Ph.D. thesis, Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, 2003, 130 pp.

PUTNOVÁ, L., KOLAŘÍKOVÁ, O., KNOLL, A., DVOŘÁK, J.: Association study of osteopontin (SPP1) and estrogen receptor (*ESR*) genes with reproduction traits in pigs. Acta univ. agric. Et silvic. Mendel. Brun., 2001, IL, No. 4, 69-74 pp.

ROTHSCHILD, M., JACOBSON, C., VASKE, D., TUGGLE, C., WANG, L., SHORT, T., ECKARDT, G., SASAKI, S., VINCENT, A., MCLAREN, D., SOUTHWOOD, O., van der STEEN, H., MILEHAM, A., PLASTOW, G.: The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proceedings of the National Academy of Sciences of the USA, 1996, 93, 201-5.

ROTHSCHILD, M. F., VASKE, D. A., TUGGLE, C. K., MESSER, L. A., MCLAREN, D. G., SHORT, T. H., ECKARDT, G. R., MILEHAM, A. J., PLASTOW, G. S., SOUTHWOOD, O. I., van der STEEN, H. A. M.: Estrogen receptor locus is a major gene for litter size in the pig. In: Proceedings of the European Association of Animal Production, Prague, 1995.

SHORT, T. H., ROTHSCHILD, M. F., SOUTHWOOD, O. I., MCLAREN, D. G., de VRIES, A., van der STEEN, A., ECKARDT, G. R., TUGGLE, C. K., HELM, J., VASKE, D. A., MILEHAM, A. J., PLASTOW, G. S.: Effect of the estrogen receptor locus in reproduction and production traits in four commercial pig lines. Journal of Animal Science, 1997, 75, 3138-42.

SOUTHWOOD, O. I., van der STEEN, H. A. M., MILEHAM, A. J., CUTHBERT-HEAVENS, D.: Evaluation of the estrogen receptor (*ESR*) gene in Meishan synthetic and Large White pigs. In: Proceedings of the European Association of Animal Production, Prague, 1995.

STEINHEUER, R., DROGEMULLER, C., HAMANN, H., DISTL, O.: Estimation of candidate gene effects on number of piglets born alive for German Landrace sows and boars. Zuchtungskunde, 2002, 74 (4), 276-287.

VAN DER LENDE, T., KNOL, E. F., VAN RENS B.

T. T. M.: New developments in genetic selection for litter size and piglet survival. Thai Journal of Veterinary Medicine, 2002, 32, 33-46.

VAN RENS, B. T. T. M.: Physiological aspects of two candidate genes for litter size in pigs. Ph.D. thesis, University of Wageningen, 2001, 210 pp.

VRTKOVÁ, I., DVOŘÁK, J.: Genetic variability in the *ESR* locus in pigs the Landrace and Large White breeds kept in the Czech Republic, Czech J. Anim. Sci., 2001, 46, 185-187 pp.

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