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Short Report

Unexpected high frequency of the stress-susceptibility conferring *RYR1* T allele in a city forest wild boar population*

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An opinion prevails, although supported by only a few reports that the RYRI T (skeletal muscle ryanodine receptor) point gene mutation (C>T transition) linked to stress-sensitivity rarely occurs in the wild boar (*Sus scrofa scrofa*) – ancestor of the domestic swine. Consequently, the wild boar has been considered genetically resistant to stress. The present study determined the frequency of

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the *RYR1* C>T mutation in a population of wild boars inhabiting the Gdańsk city forest (Poland). Blood samples from the hunted boars were analysed for the evidence of the RYR1 T allele. Among the 29 genotyped animals, there were 19 (65%) stress-sensitive recessive homozygotes (TT), 8 (28%) stress-resistant heterozygotes (CT) and only 2 (7%) stress-resistant dominant homozygotes (CC) with both alleles intact. Thus, contrary to the reported observations, it would appear that the wild boar population of the Gdańsk forest contains predominantly animals with at least one *RYR1* T allele (frequency q = 0.79). The finding is a striking example of potentially detrimental, invisible, bottleneck effect in a relatively robust population of wild animals.

KEYWORDS: bottleneck effect / halothane test / malignant hyperthermia / RYR1 gene mutation / stress-susceptibility, *Sus scrofa scrofa*, wild boar

Individual variability in stress-susceptibility in the wild boar (Sus scrofa scrofa), the ancestor of the domestic swine (Sus scrofa domestica), seems to arouse little research interest. In swine, the ryanodine receptor 1 gene (RYR1) mutation (C>T transition; RYR1 T allele) is associated with stress susceptibility (porcine stress sydrome – PSS) and a decrease of some parameters of meat quality (pale, soft exudative meat -PSE) and reproduction [Fujii et al. 1991, MacLennan and Phillips 1992, Tokarski et al. 1992, Houde et al. 1993, Murray and Johnson 1998, Weaver et al. 2000, Ciepielewski et al. 2013, Stratz et al. 2013]. There have been several attempts to determine the occurrence of the *RYR1* mutation in wild boar. Apart from extremely rare heterozygotes (CT), only stress-resistant dominant homozygotes (CC) were reported [Andersson-Eklund et al. 1998, Müller et al. 2000, Ernst et al. 2003, Kuryl et al. 2004]. Available data thus suggest that wild boars are rarely carriers of the RYR1 T alleles that in recessive homozygotes (TT) result in stress susceptibility and, therefore, are inherently resistant to stress. In the present study, 29 individuals from a population of approximately 200 wild boars inhabiting the Gdańsk city forest (Poland) were genotyped. The migration possibilities of animals are affected due to habitat fragmentation, a population bottleneck may occur and, thus, an increase in frequency of recessive RYR1 T alleles.

Material and methods

Wild boar habitat

The study was carried out in the Gdańsk city forest (District) in Pomerania, Poland. The District occupies 20690 ha, with wooded areas accounting for 19920 ha, and the whole territory being recognized as a protected forest. However, almost half (1.1 mln) of the human population of the Pomerania province lives within the administrative boarders of the District. Such a location generates various problems, the prevailing being habitat fragmentation and damage to the forest floor due to human treading. Blood samples were collected from wild boars inhabiting area No 37A of the District (encompassing forests in Gołębiewo, n=1; Matemblewo, n=18; Renuszowo, n=2; and Sopot, n=8) located between the Gdańsk-Sopot-Gdynia ring highway and a compact urban development. The wild boar population which lives there (about 200 individuals) is under strong anthropopressure and has limited possibility of migration. The entire district is under the management of the state forest service and there have been no reports of feral domestic pigs (*Sus scrofa domestica*). No approval was required from the local animal ethics committee for the study because blood samples were collected during a routine cull by the forest wildlife management.

Blood samples collection and genotyping

Seventeen blood samples were collected in December 2009, nine in January 2010 and three in February 2010 (25 females and 4 males). The samples were taken with a syringe from the heart ventricle immediately upon death, transferred to sterile heparinised Vacutainer tubes (Becton Dickinson, USA), labelled and frozen at -25° C. The samples were subjected to a routine genetic test based on the detection of point mutation in the *RYR1* gene (*RYR1* g.1843C>T). Genotyping was carried out by a laboratory of the Pig Hybridization Center of the National Research Institute of Animal Production in accordance with the previously described procedures [Fujii *et al.* 1991, Otsu *et al.* 1992, Ciepielewski *et al.* 2013] and it identified dominant homozygotes (CC) and heterozygotes (CT) defined as resistant to stress, and recessive homozygotes (TT) defined as sensitive to stress.

Results and discussion

The study determined the allelic frequency at the *RYR1* locus in an isolated population of wild boars from Gdańsk forest district. Majority of genotyped animals, that is 19 individuals out of 29 (65%), were stress-susceptible recessive homozygotes (TT), with both *RYR1* alleles mutated (Tab. 1). Stress-resistant carriers of the defective *RYR1* gene, that is heterozygotes (CT), accounted for 28% (8 boars). Two boars (7%) were stress-resistant with both alleles intact (dominant homozygotes, CC). Thus, the vast majority of wild boars in the Gdańsk forest district are, paradoxically, animals with the *RYR1* T allele linked to stress-susceptibility (frequency q=0.79).

Our findings contradict few reports that indicate absence of the RYR1 T allele in

Table 1. Allele C and T as well as genotype *RYR1* frequenciesamong 29 wild boars of the Gdansk forest district,Poland. CC, wild type (no mutation) homozygotes –stress resistant; CT, carriers of the defective *RYR1*gene (*RYR1* g.1843C>T) – stress resistantheterozygotes; TT, homozygotes with both allelesmutated – stress susceptible

Allele/genotype	Number	Frequency (q)
С	12	0.21
Т	46	0.79
CC	2	0.07
CT	8	0.28
TT	19	0.65

wild boars. Müller *et al.* [2000] genotyped 10 European boars from Germany, Kurył *et al.* [2004] genotyped 90 boars from North-East Poland, Ernst *et al.* [2003] genotyped 2 subspecies of wild boar (*Sus scrofa scrofa,* n=67 and *Sus scrofa attila,* n=42) from Germany and Czech Republic and reported only CC homozygotes. In fact, the wild boar has been long considered as free of *RYR1* T allele and this phenomenon was one of the most important criteria considered in the choice of breeds for the generation of the reference family created for quantitative trait *loci* (QTL) mapping in pigs. However, it was also reported that one of European wild boar used for arrangement of the Nordic reference family for QTLs mapping had been a heterozygote (CT) at the *RYR1 locus* [Andersson-Eklund *et al.* 1998]. The finding suggests that among wild boars, CT heterozygotes do occur.

In domestic pigs, the recessive *RYR1* T homozygotes display PSS and PSE but also malignant hyperthermia susceptibility (MHS) and impaired adaptation to sudden temperature changes [Topel *et al.* 1968, MacLennan and Phillips 1992, Houde *et al.* 1993, Weaver *et al.* 2000, Prunier *et al.* 2010]. Effective thermoregulatory behavior is of crucial importance in *Sus scrofa* [Swiergiel and Ingram 1986, Swiergiel 1987] and survival rate of the homozygous recessive offspring of the wild boar heterozygotes may be affected. It could be assumed that if the *RYR1* C>T mutation occurred in wild boars, survival of the stress-sensitive animals and transmission of this mutation to their offspring would be unlikely. Still, our results clearly demonstrate that the increased frequency of *RYR1* T allele occurred in the wild. Different mechanisms may account for this remarkable genetic drift.

First of all, it is should be recalled that the lethal effects of the RYRI C>T mutation occur mainly in extremely stressful situations of transport, overcrowding and overheating [Murray and Johnson 1998]. Our previous studies revealed that stresssusceptible pigs displayed a number of advantageous features of their immune system, compared to stress-resistant animals [Tokarski and Borman 1988, Borman et al. 1992a, 1992b, Tokarski et al. 1992, Stojek et al. 2006, Ciepielewski et al. 2013]. Finally, already many years ago Dantzer and Mormede [1983] pointed out that behavioral and physiological responses of stress-sensitive pigs - their higher locomotor activity than in stress-resistant pigs, better adaptation to conflict, better coping with "frustration". strong herd instinct, good appetite, and interest in the environment - did not indicate any impairments in adaptive functioning. In some circumstances, for example in partially urban forest environment, the benefits of having certain behavioral traits or specific "behavioral profile" may perhaps outweigh biological risks associated with increased susceptibility to stress under other conditions. This mechanism may explain apparently normal performance and survival of the small population inhabiting a limited area under strong anthropopressure, but displaying a very high frequency of the *RYR1* C>T mutation. In the Gdańsk forest, an additional mechanism, a population bottleneck, may have produced a radical change in RYR1 alleles frequency. The immediate effect of a population bottleneck is to decrease genetic diversity, promoting the effects of stochastic genetic drift over natural selection [Keller and Waller 2002].

In the long-term, repeated population bottlenecks can severely decrease population fitness: deleterious alleles are able to accumulate, especially where the time interval between bottlenecks does not allow for the generation of new alleles through mutation [Reed and Frankham 2003]. Eventually, in a true population bottleneck, the odds for survival of any member of the population are purely random, and are not improved by any particular genetic advantage (as it may seem for a moment with *RYR1* T mutation). Then, with an adverse or rapid change in living conditions, such as disease, climate change, shift in the available food source, or even a necessity of animals relocation, an entire local animal (wild boar) population, even though seemingly robust and numerous, will be endangered.

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