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THE RAPD ANALYSIS OF GENETIC VARIABILITY IN THE GRANARY WEEVIL (*SITOPHILUS GRANARIUS* L.) POPULATIONS

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Abstract: Granary weevil (*Sitophilus granarius* L.) belongs to primary stored-product pests and causes extensive economical losses by reducing quantity and quality of the grain and wheat products. The morphological diversity within each of three most common weevil species: *S. granarius*, *S. oryzae* and *S. zeamais* is high. All three species can also feed on different types of grain.
The aim of our study was to check the range of molecular diversity between ten populations of *S. granarius* derived from different sources in Poland, Europe and USA and to compare results with those obtained for some populations of *S. oryzae* and *S. zeamais*. For phylogenetic analysis we applied the RAPD technique, which provides DNA markers dispersed throughout the whole genome and are easy to collect and analyse. The phylogenetic analysis of obtained results revealed the high similarity between all populations of granary weevil, European as well as American ones and, simultaneously, considerable diversity between granary weevil and rice and maize weevils.

Key words: *Sitophilus granarius*, genetic variability, RAPD

INTRODUCTION

The granary weevil, *Sitophilus granarius* (L.) is a little (2,5–4 mm), reddish brown to dark brown beetle infesting stored grain, mainly wheat grain. Together with two other species, rice (*S. oryzae*) and maize weevils (*S. zeamais*) it belongs to the most destructive pests of grains, seeds, and grain products stored in elevators and bins. These

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pests are widely distributed throughout the world and can feed on wheat, corn, oats, barley, sorghum, Kaffir seed and buckwheat. Granary weevil is not capable to fly, therefore it is confined to stored grain and is primarily transported by man, whereas infestation with rice and maize weevils, which are able to fly, could develop on a field prior to harvesting (Levinson and Levinson 1994).

The origin of granary weevil remains enigmatic, although it had been found in ancient Egyptian tombs from 3rd millennium BC (Levinson and Levinson 1994) and it presumably spread from there throughout Northern Europe during the Roman period. Afterwards, granary weevil was probably brought to USA from Europe with the grain (Calvin 2001). The most likely origin of rice weevil is India, now it occurs in subtropical and tropical climate: Australia, Mexico, Africa, India, South-West Asia. It could had been introduced to Poland with the transport of rice and maize. Maize weevil occurs in tropical and subtropical regions such as: India, middle and South Asia, Mexico, southern USA, Argentina and China (Nawrot 2006).

Polymerase chain reaction-based random amplified polymorphic DNA (PCR-RAPD) technique had been applied as a technique suitable for studying genetic diversity among populations in early 90's of recent century (Huff *et al*. 1993) and since then have been commonly used to estimate genetic relationship within species (Orti'z-Dorda *et al*. 2005). In present study the technique was employed for quantifying RAPD variation among ten populations of *S. granarius*, simultaneously analyzing dispersion of markers in two populations of *S. zeamais* and one population of *S. oryzae*, used as outgroups.

MATERIALS AND METHODS

Biological material

Adults of the granary weevil used in experiments came from our laboratory colony maintained on whole wheat kernels (winter variety KANCLER) at 24°C and 65–70% relative humidity and were kindly provided by various institutions from Poland, Germany, Denmark, France and USA. Two populations of maize weevil came from Germany and Italy and the rice weevil came from our laboratory colony (Tab. 1).

DNA isolation

From each population 5–10 insects were selected and total DNA isolated from whole weevils using DNeasy® Tissue Kit (Qiagen, Valencia, CA, USA) after overnight digestion with Proteinase K. The integrity and purity of DNA was checked during electrophoresis (Sambrook *et al*. 1989) and the DNA concentration was measured using spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

RAPD analysis

For the RAPD analysis of *Sitophilus* populations 20 primers have been applied. Sequences of primers and annealing temperatures for the primers finally used for analysis are grouped in table 2.

PCR reactions were carried out in Eppendorf Mastercycler in the final volume of 10 µl. The reaction mixtures contained 50 ng DNA, 1 µM of the primer, 200 µM dNTP (Novazym), 2mM $MgCl_2$, 0.2 U of Platinum®*Taq* polymerase in 1x Rxn buffer (Invitrogen, Carlsbad, CA, USA). The samples were amplified for 40 cycles. Each cycle

Table 1. Origin and IDs of *Sitophilus* ssp. populations used in present study

The weevil's species	Origin	Type of culture	Population ID
S. granarius	Institute of Plant Protection – National Research Institute, Poznań, Poland	laboratory	SgP0
S.granarius	Uscikowo Oborniki Wlkp., Poland	wild	SgP1
S. granarius	University of Warmia and Mazury in Olsztyn, Poland	laboratory	SgP10
S. granarius	Kraków, Poland	wild	SgP11
S. granarius	Brzeg, Poland	wild	SgP14
S. granarius	Fajslawice, Lublin, Poland	wild	SgP15
S. granarius	Julius Kuehn Institute, Berlin, Germany	laboratory	SgGe
S. granarius	*LND*S-QUALIS, Villenave D'ornon Cedex, France	laboratory	SgFr
S. granarius	University of Aarhus, Lyngby, Denmark	laboratory	SgDe
S. granarius	Biological Research Unit USDA-ARS, Manhattan, Kansas, USA	laboratory	SgUS
S. oryzae	Institute of Plant Protection – National Research Institute, Poznan, Poland	laboratory	So
S. zeamais	University of Molise, Campobasso, Italy	laboratory	SzIt
S. zeamais	Julius Kuehn Institute, Berlin, Germany	laboratory	SzGe

consisted of the following steps: denaturation at 95°C for 1 min, annealing at 32–48°C for 30 s (Tab. 2), primer extension at 72°C for 2 min 30 s. After the last cycle, final elongation was carried out for 10 min.

PCR products were separated in 1,2 % agarose gels (NuSieve Agarose, Cambrex) stained with ethidium bromide and visualised under UV light. DNA bands were counted and their molecular weigth was estimated using UviBand software (UVITEC, Cambridge, UK).

Statistical analysis

Statistical analysis was performed using MrBayes program (Huelsenbeck and Ronquist 2001). The sequence dataset was modeled using the Restriction Site (Binary) Model corresponding to the JC model. Features were encoded as informative characters. A Metropoliscoupled Markov-chain Monte-Carlo analysis was preformed with 1.000.000 generations and two runs without heated chains. The Markov chain was sampled every 100th generation. Convergence of runs was confirmed by average standard split deviation factor that fell under the recommended value of 0.01 and reached 0.006. The final tree was obtained after removing the first 25% of samples. Obtained phylogenetic tree was visualised and edited using TreeView program (Page 1996).

Table 2. Primers used in RAPD analysis in the present study

Primer name	Sequence 5′→3′	Annealing temperature (Ta) [°C]
OPO02	ACGTAGCGTC	46
PSM5	GAGGGTGGCGGTTCT	44
OPC5	GATGACCGCCACGTAGCGTC	48
OPE04	GTGACATGCC	34
OPE07	AGATGCAGGC	36
OPE08	TCACCACGGT	36
OPE10	CACCAGGTGA	34
OPE14	TGCGGCTGAG	34
A04	AATCGGGCTG	36
C02	GTGAGGCGTC	34
D05	TGAGCGGACA	36
D13	GGGGTGACGA	36
F06	GGGAATTCGG	34
J20	AAGCGGCCTC	34
2	ATGGATCCGC	34

RESULTS AND DISCUSSION

The use of PCR-RAPD has been proposed for applications regarding differentiation between insect species and strains (Hoy 2003), determination of the origin of infestation during the transport through marketing channels (Dowdy and McGaughey 1993) and phylogenetic analyses (Taberner *et al*. 1997). The technique allows for detection of small differences in the genomes of stored product insects at the species as well as geographical populations level (Fleurat-Lessard and Pronier 2006).

The aim of our study was to assess the variability level between geographically isolated Polish populations of *S. granarius* on the basis of their molecular features and to inquire whether the differences between Polish and other European populations are significant. Two populations reared in isolation for decades (laboratory colonies from Poznań and Berlin) were used as well as some wild populations. The strongly geographically isolated USA population was chosen for a comparison to show the tendency in variability, if such would be ascertained.

Out of tested 20 primers, 15 gave informative results that were used in further analysis. On the basis of analysis of RAPD results in UviBand the most clear and informative bands of 500–3000 bp length were selected for statistical analysis (Fig. 1). Results were assembled as 178 binar features for 13 analysed populations and computed in MrBayes program.

Resulting phylogenetic tree was supported with high bootstrap values (Fig. 2.) and confirmed closer relationship between rice and maize weevils. The populations from Europe and USA seem to share equal level of diversity, apart from the laboratory colony from Germany and our Institute's laboratory colony originating from a few mixed populations, one of them derived from Germany. Close relationship between

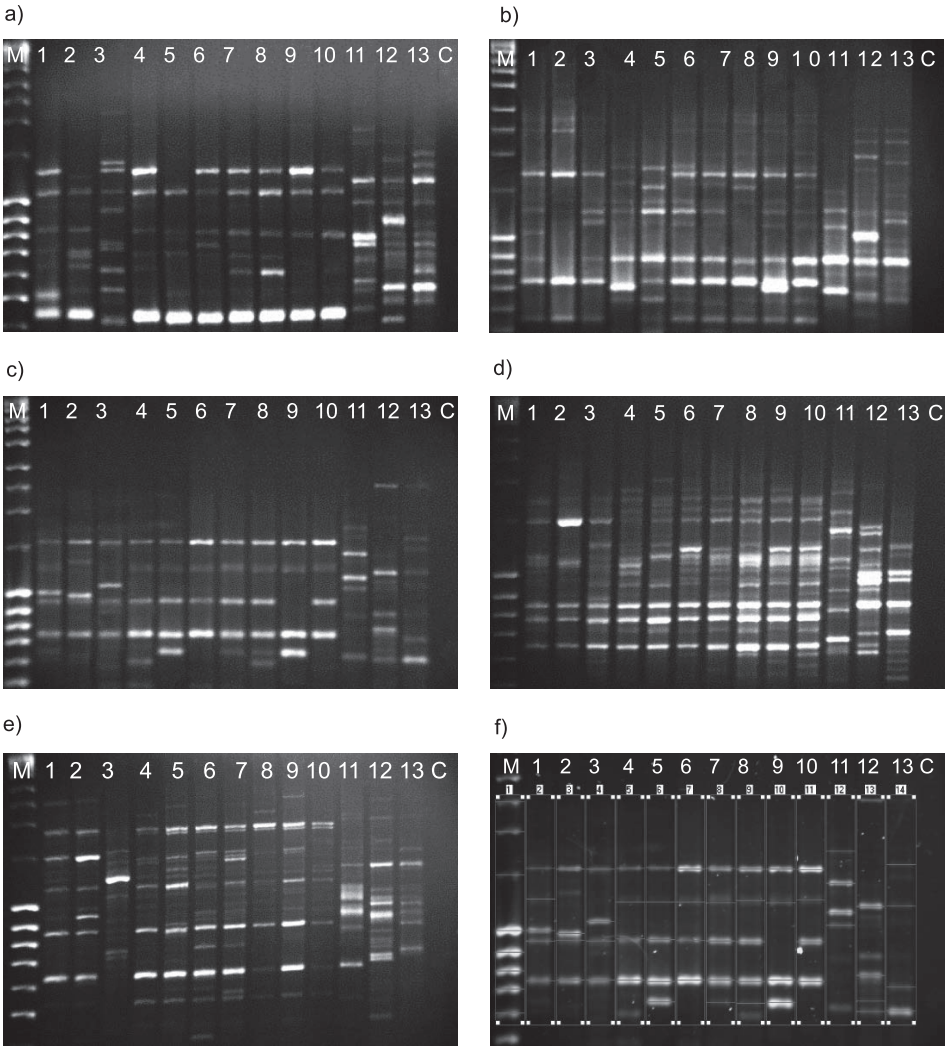

Fig. 1. Exemplary results of electrophoresis of PCR products with primers
a) PSM5 b) OPC5 c) OPO02 d) F06 e) OPE14
f) analysis of results for primer C02 in UviBand software

M – mass marker
1 – SgP0
2 – SgGe
3 – SgFr
4 – SgDe
5 – SgP1
6 – SgP10
7 – SgP11
8 – SgUS
9 – SgP14
10 – SgP15
11 – So
12 – SzGe
13 – SzIt
C – negative control

European and American populations was confirmed on the molecular level, affirming the hypothesis of European origin of granary weevil population from USA. The difference between two laboratory colonies (from Polish Institute of Plant Protection and the Institute for Stored Product Protection in Berlin) and the rest of European

populations might result from the long isolation of these populations (in the case of German population 70 years) and inbreeding. Generally, the variation within *S. granarius* specimen is low, probably due to the lack of evolutionary pressure and similar living conditions (stored grain).

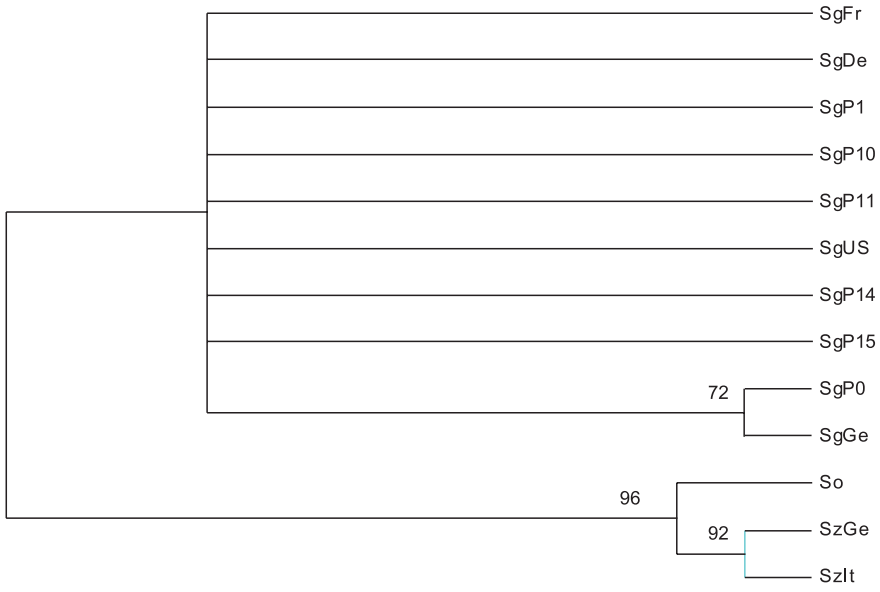

Fig. 2. Phylogenetic tree of *Sitophilus* ssp. populations obtained after statistical analysis of RAPD results showing close relationship between *Sitophilus granarius* populations taken to the analysis

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POLISH SUMMARY

BADANIE ZRÓŻNICOWANIA GENETYCZNEGO POPULACJI WOŁKA ZBOŻOWEGO (*SITOPHILUS GRANARIUS* L.) METODĄ RAPD

Wołek zbożowy (*Sitophilus granarius* L.) jest ważnym ekonomicznie szkodnikiem magazynowym redukującym zarówno ilość, jak i jakość ziarna pszenicy oraz produktów spożywczych z niej uzyskanych. Zróżnicowanie morfologiczne pomiędzy trzema powszechnie występującymi wołkami: ryżowym – *S. oryzae*, kukurydzianym – *S. zeamais* oraz zbożowym jest znaczne. Każdy z trzech gatunków żywi się na różnych typach ziarna. Celem naszych badań było określenie zakresu zróżnicowania genetycznego pomiędzy populacjami *S. granarius* pochodzącymi z różnych źródeł w Polsce, Europie i USA oraz porównanie otrzymanych wyników z tymi uzyskanymi przy analizie wybranych populacji *S. oryzae* i *S. zeamais*. Do badań filogenetycznych zastosowano technikę RAPD, która umożliwia poszukiwanie markerów genetycznych rozproszonych w całym genomie i oparta jest o losową amplifikację polimorficznego DNA. Analizy filogenetyczne otrzymanych wyników wykazały wysoki poziom podobieństwa wszystkich populacji wołka zbożowego zarówno europejskich, jak i amerykańskiej, jednocześnie wykazując znaczne zróżnicowanie w stosunku do innych gatunków wołka ryżowego oraz kukurydzianego.