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Genome size and base composition in *Oryzaephilus surinamensis* (Coleoptera: Sylvanidae) and differences between native (feral) and silo pest populations in Israel

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ABSTRACT

In this study, flow cytometry was used for assessing and comparing the genome size (GS) and the whole genome base composition (AT/GC ratio) of the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.). In addition, the presence and frequency of endosymbiotic *Wolbachia* bacteria was studied. The haploid GS was estimated to lie within the range of 151.5–154 Mbp in *O. surinamensis*, making it the smallest value of haploid GS known among beetles. Furthermore, it was found that in eight silo pest populations GS was significantly smaller than in eight feral (native) populations obtained from fallen oak acorns. The ability of *O. surinamensis* to colonize different habitats globally could be connected with an unusually AT-rich (for an invertebrate) genome (AT-base content ranging from 68 to 76%). Native (feral) populations of *O. surinamensis* appear to have genetically diverged from the storage-pest populations tested. Larvae of pest origin survived better than larvae of native (feral) origin under laboratory conditions, which resembled silo conditions more than natural habitats.

1. Introduction

The warm-adapted cosmopolitan storage pest *Oryzaephilus surinamensis* (L.) is 2.5–3.5 mm in length, has heteromorphic sex chromosomes (Robertson, 1959), and a life cycle lasting from three to ten weeks (Richara, 2003). In Israel, in addition to storage-pest populations, native (perhaps feral) populations are present in fallen acorns of the East Mediterranean oak *Quercus calliprinos* Webb (Sharaf et al., 2008). The comparison of the relative genome sizes between one native population and one pest (silo) population documented intersexual differences in genome size associated with the presence of heteromorphic sex chromosomes in *O. surinamensis* (Robertson, 1959), and a smaller genome size present in the silo population than in the feral (native) one (Sharaf et al., 2008). To confirm these results, we compared and contrasted genome sizes among several additional native and pest populations. Furthermore, we tested whether the whole genome and the base composition was in the range expected from the beetle's small body size, holometabolic development, short life cycle and ability to quickly colonize new food resources. The differences between pest and native (feral) populations in infection rates by *Wolbachia* bacteria and in larval survivorship during development in the laboratory were also investigated.

2. Material and methods

A flow cytometer Partec CyFlow ML (equipped with a 100 mW laser Cobolt Samba) was used to estimate the genome DNA content and the whole genome base composition in combination with a Partec PA-1 cytometer (equipped with a Mercury HBO lamp). Sample preparations were carried out in a two-step procedure (Otto, 1990). The following internal standards were used: one individual each of *Raphanus sativus* 'Saxa' (2C = 1.11 pg (Doležel et al., 1998)), *Oryza sativa* subsp. *japonica* 'Nipponbare' and *Arabidopsis thaliana* 'Columbia', or an isofemale line of *Drosophila melanogaster* (Table 1). The reference standard (1 cm^2 of leaf blade or half of one head of *D. melanogaster*) was chopped together with a head of *O. surinamensis* (Nardon et al., 2003) for about 20 s in a Petri dish containing 1 ml of ice-cold Otto I buffer (4.2 g citric acid monohydrate + 1 ml 0.5% Tween 20 adjusted to 200 ml and filtered through a 0.22 μm filter). Then, another 1 ml of Otto I buffer was added and the solution was filtered through a nylon cloth (50 μm mesh size) before being divided equally into two tubes. For DNA

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Table 1
Estimated haploid GS and AT content in *Oryzaephilus surinamensis* in comparison with standards.

Standard	Standard: 1C (Mbp)/AT-base content	*O. surinamensis* : 1C (Mbp/Pg)	Dye factor[a]/ Binding length	*O. surinamensis*: AT base content
Oryza sativa	389[b]/0.570[a]	151.4/0.155	1.751/4	0.685
O. sativa	–/0.564[b]	–	1.751/4	0.677
Arabidopsis thaliana	157[d]/0.609[a]	153.2/0.157	1.421/4	0.684
A. thaliana	–/0.653[b,c]	–	1.421/4	0.736
D. melanogaster	175[e]/–	154.1/0.158	–	–
Raphanus sativus	–/0.609[a]	–	1.489/4	0.695

[a] Barow and Meister, 2002.
[b] Matsumoto et al., 2005.
[c] Arabidopsis Genome Initiative, 1997.
[d] Bennett et al., 2003.
[e] Adams et al., 2000.

staining, 1 ml of Otto II buffer (0.4 M disodium hydrogen phosphate dodecahydrate) including AT-selective DAPI (4′,6-diamidino-2-phenylindole; 2 μg/ml final concentration) for measurement by PA-1, or base-independent PI (propidium iodide, 25 μg/ml final concentration) for measurement by CyFlow ML, was used for each tube. Each sample was measured five times on different days and the average value was included in the statistical analysis.

The presence of *Wolbachia* was tested by using genus-specific primers for the 16S rDNA gene in a PCR (Heddi et al., 1999). Studied specimens were homogenized in 40 μl lysis buffer (Frohlich et al., 1999), followed by incubations at 65 °C for 15 min and at 95 °C for 10 min. Reactions were carried out in a 25 μl volume containing 2 μl of the template DNA lysate, 10 pM of each primer, 0.2 mM dNTP's, 1 X RedTaq buffer and one unit of RedTaq DNA polymerase 108 (Sigma).

Survivorship was tested on larvae isolated from the progeny of a beetle culture established from 200 to 300 specimens collected at lower Nahal Oren, Mt. Carmel (native (feral) population) and from a silo near Haifa (pest population). First instar larvae isolated from the progeny were kept until the end of their development in 2 ml tubes containing 0.5 g of crushed barley seeds and yeast for nutrition. Both the beetle culture and tubes with larvae were incubated at 28 °C.

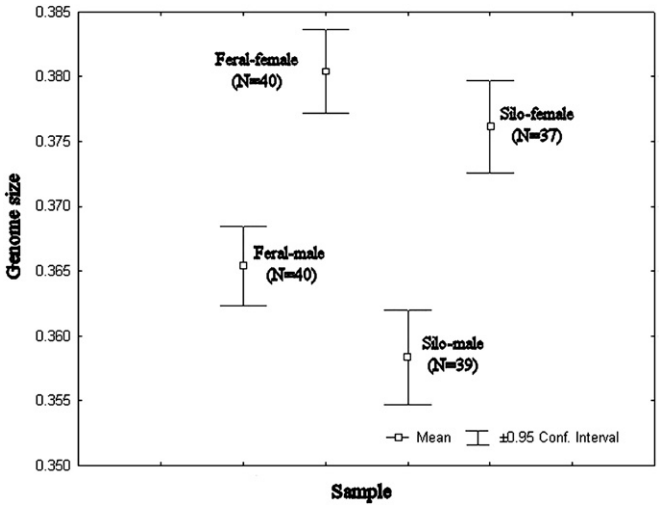

Fig. 2. Comparison of the relative GS differences between eight pooled feral population samples and eight pooled silo samples of *Oryzaephilus surinamensis* in Israel. The figure also shows smaller GS in pooled silo samples than in pooled feral samples.

3. Results and discussion

The use of flow cytometry revealed a haploid GS in *O. surinamensis* ranging between 151.5 and 154.1 Mbp (Table 1). These numbers are below the estimated GS in the standards used (Table 1, Fig. 1), and in 184 beetle species so far studied (Gregory, 2008).

The method used was sensitive enough to detect an intersexual difference of 4% in the native populations (pooled samples of eight populations $x_{(female)} = 0.38$, $x_{(male)} = 0.365$, Mann–Whitney U test, $Z = -5.67$, $P < 1.10^{-6}$) and an intersexual difference of 4.8% in the silo pest populations (pooled sample of eight populations, $x_{(female)} = 0.376$, $x_{(male)} = 0.358$, Mann–Whitney U test, $Z = -5.87$, $P < 1.10^{-6}$). The intersexual differences have previously been shown in *O. surinamensis* (Sharaf et al., 2008) and are associated

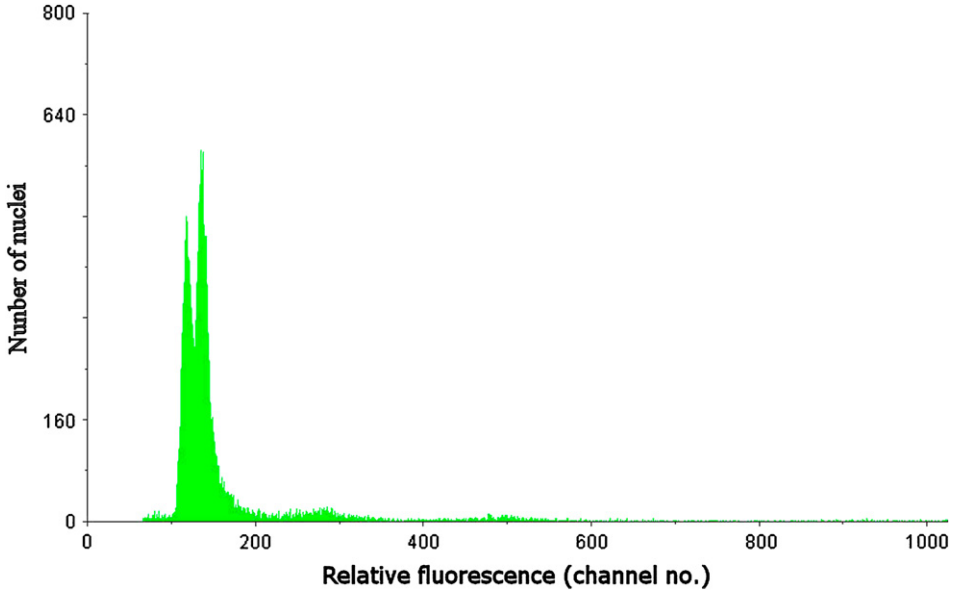

Fig. 1. Flow cytometry relationship between genomes of *Oryzaephilus surinamensis* (left peak) and *Drosophila melanogaster* (right peak) in PI staining.