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DNA polymorphism detection in *Tribolium castaneum* (HERBST) (Coleoptera: Tenebrionidae): potential use in stored product pest management

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Abstract: Different pairs of oligonucleotide primers have used for the detection of DNA polymorphism between *Tribolium castaneum* strains. Amplification of a major DNA fragment and two minor was observed in all strains tested excepted for one strain from France which present only two major bands (respectively at 800 bp and 880 bp). Arbitrarily, the major band amplified from a DNA sample of the strain originating from the Philippines was choosen as a probe (sequence of 487 bp in length). The combination of this probe and restriction enzymes permitted the discrimination between strains. Our results show that RAP marker of Tribolium castaneum could be used to study its population genetics.

Key words: Tribolium castaneum, polymorphism, PCR.

Introduction

In field conditions, insect species in the stored product ecosystem evolve and species diverge for a great variety of physiological, biochemical and morphological characters when they are subjected to similar selection pressures (temperature, humidity, presence of pesticide or nutrition substrate).
The stored product ecosystem which is very closed and principally controlled by man is a wonderful model to understand the introduction and the movement of pest populations.
As cereal surplus are becoming very important in developed countries, there is a urgent need to reconsider strategies of grain storage. Presence of pest species in silos where grain are warehoused for a long periods is a acute problem. Insects and mites provoke heavy weight losses and affect the quality of alimentary products.
Moreover, intensive use of pesticides such as phosphin and malathion has triggered the development of resistant strains of pest. In this context, control methods of pest population have to become more precise. That means a better integration between biological, ecological and ethological parameters knowledges of animals which have become adapted to man-made ecosystems.
For these reasons, we have undertaken the study of the dispersion and the movement of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) strains.
Tribolium castaneum is a cosmopolitan pest in cereal mills and wherever flour is stored. Its region of origin is a unknown as its natural habitat outside man's activities. The rearing of this species is very easy and cheap in the laboratory. The developmental time is fairly short (25 days at 32°C and 70% relative humidity) and it reproduces for the whole twelve-month without diapause.
Until now, discrimination between strains in this species has often been achieved thanks to isozymes polymorphisms. This rather simple method sometimes gives a poor resolution, and depends, for some enzymes, on the ontogenesis and requires sufficient levels of intraspecific variations.
These limitations could be overcome by the use of strain-specific or strain polymorphic DNA probes. Indeed, anonymous low copy number genomic clones, multiple tandem-repetitive DNA sequences or hypervariable minisattelites have been commonly used, as DNA probes, to visualize polymorphisms in many species (Martin e*t* al., 1983; Jeffreys e*t* al., 1985; Kirchhoff, 1988; Tautz, 1989). Recently, Black IV e*t* al. (1992) have identified genetic polymorphism among and within aphids. Here we report the use of random anonymous probes (RAP), produced by Polymerase Chain Reaction (PCR) to show RFLPs between Tribolium castaneum. RAP have been first described by Williams e*t al.* (1991) and since then have been used to generate markers in many organisms like bacteria, plants and mammals.

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Results

Probe generation

For the detection of DNA polymorphism between *Tribolium castaneum* strains, we have used different pairs of arbitrary oligonucleotide primers, as suggested by Willians e*t* al. (1991).
Analysis of DNA products obtained by PCR amplification, with our set of primers, revealed several discrete bands on agarose gel (data not shown). We could observe amplification of a major DNA fragment and two minor in all strains tested excepted for one strain (strain Bordeaux) which present only two major bands (respectively at 800 bp and 880 bp) (Fig. 1).
Arbitrarily, the major band amplified from DNA sample of *Tribolium castaneum* strain, originated from Philippines, was choosen as a probe. Its sequence was 478 bp in length (Fig. 2) and did not reveal significative homology with the sequences data contained in the GeneBank.

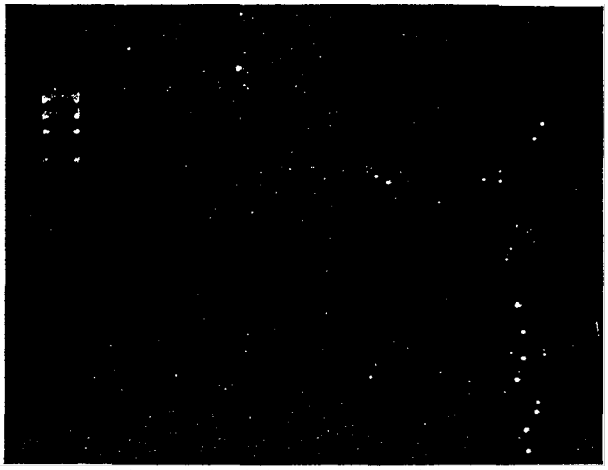

Figure 1 Amplification of Tribolium DNA. From five strains of Tribolium castaneum, DNA was amplified using m1 and m2 primers. Amplification products were resolved by electrophoresis in a 1% agarose gel which have been stained with ethidium bromide and photographed. Moiecular weight markers (Kilobases, kb) are as indicated, lane 1, T.c.s. Bordeaux; lane 2, T.c.s. Botswana; lane 3, T.c.s. CTC 12; lane 4, T.cs. lnde; lane 5, T.c.s. Jamagne; lane 6, T.c.s. Philippines; lane 7, T.c.s. Nioro; lane 8, T.c.s. Tirlemont. DNA was omitted in control reaction (lane 9).

Restriction fragments analysis of Tribolium strains

Southern blots prepared from *Tribolium* strains and others species (human, bovine, bumble bees and Rhyzopertha dominica), were hybridized with the probe. In stringent (65°C) or relax conditions (50°C) only *Tribolium* DNA gave a positive signal. Among *Tribolium* strains, those originated from Bordeaux, India, Senegal were poorly recognized by the probe. More, the absence of hybridization signal with foreigners DNA, confirm the specifity of the used probe.
However, in the strains, this probe revealed numerous components in all restriction enzyme patterns. Figure 3 shows an example of RFLPs pattern. In this case, genomic DNA for all *Tribolium castaneum* strains were digested by *Kpn* I and *Sac* I. After hybridization, the probe revealed numerous fragments of various intensity and size, y.
All restriction patterns are summarized in table 1., We have taken into account only'the presence or the absence of a band, not its variation of intensity , between strains. The comparison of all restriction patterns revealed that *Tribolium castaneum* originated from Botswana is very different from all other strains. It is characterized by the presence of an 0,4 kb band in Haelll restriction pattern. The other strains CTC 12, Jamagne, Philippines and Tirlemont present two common bands: the 3,2 kb band in *Pst* I restriction pattern and the 2 kb band in the *Kpn* I/Sac I restriction pattern.
In addition, we could remark that CTC 12 and Jamagne strains are very similar and only differ for the 2,4 kb band in the *Eco* Rl/*P*sf I restriction pattern. Philippines and Tirlemont strains, were also closed to each other but differ for four bands: the 5,9 kb band in *Pst* I restriction pattern, the 4,5 kb band in *Kpn I*/Sac I restriction pattern and for the 4,2 kb and 6,6 kb bands in the *Eco* Rl / *Pst* I pattern.
Moreover, we could separate the first two strains (CTC 12 and Jamagne) from the last two (Philippines and Tirlemont) for the four bands: the 4,2 kb and 4,5 kb in the *Eco* Rl restriction pattern, the 4 kb band in *Pst* I restriction pattern, the 3,2 kb band in *Kpn* I/Sac I restriction pattern and the 2,8 kb band in *Eco RI/Pst* I restriction pattern.

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5'- TGTCCATACA CAGCTCAGCA ATCCAACAGT GGACCAACAC CTGGCGCCGT      50
    CAAACCAACC CACAAATCCA ATCATACATG TATACAAATG CTCCAGACGT     100
    GCCCTCTCTC AACCCATTCA ACTTCTCCTC AATAATCAAC AAATTCCTCA     150
    TCTCAAAACC ATCAAATACC TCGGAGTCAC CTTCTCACAC ACTTCGTTCC     200
    TTTAGCCCCC GACATTAACG AACCCTCAAA AAAGTCCGAA ACCGCGCCAA     250
    CCTTCTCTAT CTCATCCGTG GCCGCCTTTA CGGCTGCAGC AGCCCCCAAA     300
    TCTTCTTTAC ACCTACAACT CTTTCATCCG CCCCGTCATT GAATACCGTG     350
    CCCCAATCTA TGCCTCTATC CCCCTCAACC AACTTCTTCA AATCGCCTCC     400
    ACCGAACGCC GAATCCTTTC GAAAATTTTC CGACTTGACC CCAGGTACCC     450
    CTGTCATCTC ATTTACATCA CCACAGAAAG CCTTACC -3'               487
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Figure 2 Sequence of the PCR amplified fragment from DNA sample of Tribolium castaneum strain originated from Philippines. The m1 and m2 primers are underlined.

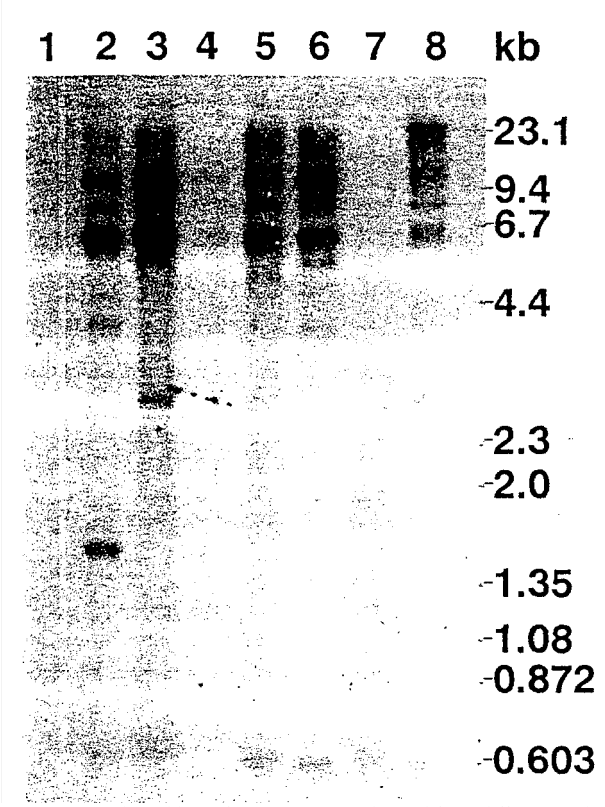

Figure 3 Southern blots of genomic DNA, derived from different Tribolium castaæum strains (T.c.s.), simultanously digested by two restriction enzymes (Kpnl/Sacl) and hybridized to the labeled probe. Lane 1, T.cs. Bordeaux; lane 2, T.c.s. Botswana; jane 3, T.c.s. CtC 12; lane 4, T.c.s. lnde; lane 5, T.c.s. Jamagne; lane 6 T.c.s. Philippines; lane 7, T.c.s. Nioro; lane 8, T.c.s. Tirlemont. λ phage DNA digested with Hindlll and fX174 phage digested with Haelll were used as molecular weight standards. κb, Kilobase(s).

Discussion

Usually, the investigation of Restriction Fragments Length Polymorphisms (RFLPs) requires the use of an homologous or an heterologous DNA probe which hybridizes to single copy or low copy number sequences. As beetles probes especially *Tribolium* ones were not immediately available, we have chosen to produce a *Tribolium* specific probe by Polymerase Chain Reaction (PCR) with arbitrary oligonucleotides primers.

In opposition to Black IV e*t* al. (1992), we couldn't obtain with PCR reaction a sufficient level of variability to directly characterize our strains, on agarose gel. However, the amplification, with m1 and m2 primers, of the same major band for all strains except one (strain Bordeaux) allowed us to check the presence of polymorphic patterns. This level of variability should be evaluated after RFLPs.

Indeed, the poor hybridization signal, in stringent conditions, with Bordeaux, Senegal and India strains probably results of major sequence differences of this sequence. To characterize these three strains, it is possible to use, as probe, the major fragment amplified in corresponding samples.

For the other strains, the combination of the probe and the restriction enzymes used, has permitted the discrimination between strains. The larger likeness in strain patterns between CTC 12 and Jamagne

strains or between Tirlemont and Philippines strains could be explained by the same geographic origin. Our results show that RAP marker of *Tribolium castaneum* could be used to study its population genetics.
It is obvious that several faces of the ecology of stored product pests have been largely neglected. For example, inside a bulk of grain in a silo, the reproductive behaviour of the pest remains obscure. In what manner young adults explore the mass of grain to select suitable places to lay eggs?. What is the impact of the reproductive strategy of a pest on the contamination of a store? To answer such questions, it could be of great importance to implement efficient methods of control and to determine whether or not control measures should be restricted to a part of the silo.

Table 1 Recapitulation of the most significant bands of Southern blots of Tribolium genomic DNA digested with different restriction enzymes alone or in combination. (+: present; -: missing).

Restriction endonucleases	Bands size (kilobases)	Strains of *Tribolium castaneum*				
		Botswana	CTC 12	Jamagne	Philippines	Tirlemont
EcoRI	4.2	-	+	+	-	-
	4.5	-	-	-	+	+
	3.2	-	+	+	+	+
	4	-	-	-	+	+
PstI	4.2	-	+	+	-	-
	5	-	+	+	-	-
	5.9	-	-	-	+	-
HaelU	0.4	+	-	-	-	-
	0.9	+	-	-	-	-
KpnI+SacI	2	-	+	+	+	+
	3.2	+	-	-	+	+
	4.5	-	+	+	+	-
	2.4	-	+	-	-	-
EcoRI+Pstl	2.8	-	-	-	+	+
	4.2	-	+	+	+	-
	6.6	+	+	+	-	+

Material and methods

Insects

Eight strains of the Red Flour Beetle were used. The strains originated from Philippines, India and Botswana were obtained from the Natural Resource Institute in Chatham (England). One aus-tralian strain (CTC 12) was obtained from Department of Zoology, Georges S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel. Two populations collected from storage places in Belgium were studied: Jamagne (Belgium), Tirlemont (Belgium); one strain in Bordeaux (France) and an other in Nioro du Rip (Senegal) were collected.

DNA isolation

Genomic DNA was prepared from 2 g adults pulverized in liquid nitrogen and resuspended in 5 ml of TE (Tris-HCI 10 mM pH 7,0, EDTA 1 mM). After centrifugation at 3000 rpm (in Sorvall HB4 rotor) for 5 minutes, 500 µl of 10% SDS and 20 µl of predigested pronase (10mg/ml) were added. The solution was gently mixed by inversion and incubated 5 hours at 37°C. Then it was gently extracted twice with 1 volume of equilibrated phenol-chloroform solution. Finally, the DNA was precipitated by the addition of 1/10 volume NaCI 4M and 2,5 volumes of ethanol 100% and recovered in 1 ml of sterile water.

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Generation of the probe

Sequences of primers

m1: 5'-GGTAAGGCTTTCTCTCGTGATGTAAAT-3'
m2:: 5'-TGTCCATACACAGCTCACCAATCCAA-3'

Amplification procedure

Amplification reactions were performed in 100 µl total volumes including 200 µM of each dATP, dCTP, dTTP and dCTP; 50 ng of genomic DNA digested by 0,5 U EcoRI (2h:37°C); 10 µl of 10x Taq buffer (Promega); 1 µg of each primer and 2,5 U of Taq DNA polymerase (Promega) on a New Brunswick Scientific TC-1 apparatus. Amplification conditions were: denaturation for 5 min. at 94°C followed by 5 cycles of primers annealing for 1 min. at 37°C, extension for 2 min. at 72°C and denaturation for 40 s at 94°C. Taq DNA polymerase was added after the first 5 min. de-naturation (hot start). After these first cycles, primers were annealed at 65°C for 1 min., extended at 72°C for 2 min. and denatured for 40 s at 94°C for 35 cycles. In final, the reaction was terminated by one cycle at 72°C for 10 min.

Probe labelling

Amplification products were separated by agarose gel electrophoresis in TAE buffer pH 8,0 (TRIS-acetate 0,04M, EDTA 1 mM). The major band was purified by Geneclean II (BIO 101 Inc.) procedure. One hundred ng of probe DNA was labelled with 100 *µCi* of α^{32}P-dCTP (Amersham) using the Multiprime labelling kit (Amerham).

Proibe cloning and sequencing

DNA sequence was determined, with the sequencing kit of Pharmacia, on both strands by the dideoxy-oligonucleotide chain-termination method (Sanger ef *at.,* 1977) after subcloning of the purified DNA fragment into a Bluescript KS+ plasmid (Stratagene), as described by Sambrook, J., Fristch, E. F. and Naniatis, T. (1989). Nolecule cloning manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (2nd edition).

Restriction fragment analysis

Restriction fragments were analyzed by Southern blotting. *Tribolium* DNA samples were separately digested by the following restriction enzymes (Amersham): Hae III, Eco Rl, Pst I, Kpn 1 + Sac I and Pst I + Eco Rl (using the conditions recommanded by the dealer). The restriction fragments are separated on 1% agarose gels (2 µg DNA/lane) run for 16 hours at 35 V. DNA was transferred on nylon membrane (Hybond N+, Amersham) by the Southern technique as described by Sambrook e*t* al. (*l*1989).
The membranes were prehybridized 3 hours at 65°C in 3xSSC (1M NaCI/0,1M sodium citrate, pH 7,0/0,1% ficoll/0,1% polyvinylpyrrolidone/ 0,1% bovine serum albumin)/0,5% sodium dodecyi sulfate (SDS)/100 µg/ml of denatured sheared salmon sperm DNA. Hybridization was done in the same solution with the heat-denatured labelled probe for 16 hours at 65°C. The membranes were washed 2x30 min. in 2x SSC/0,1% SDS followed by 2x 30 min in 0,2x SSC/0,1% SDS at 65°C and exposed to X-Omat AR5 film (KODAK) for 1 day.

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