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THE USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS FOR STUDIES OF GENETIC VARIATION IN POPULATIONS OF *COCCINELLA SEPTEMPUNCTATA* IN BELGIUM

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ABSTRACT

The movement and dispersion of *Coccinella septempunctata* and its efficacy as aphid control agent over large areas is not really understood because of the difficulty in identifying the origins of predators. To quantify the genetic diversity within the species and monitor the spatial foraging, populations were sampled from Belgium and analysed for RAPD DNA variation. Twenty decamer primers generated more than hundred polymorphic RAPD bands and pairwise distances were calculated between populations according to Nei and Li, then used to construct a radial neighbour-joining dendrogram and examine intra- and inter-population variance coefficients, by analysis of molecular variation (AMOVA). This study shows that while a number of factors can complicate the use and interpretation of RAPD fragments as genetic markers, RAPD analysis can be a valuable technique for studies of intra-specific genetic variation in *C. septempunctata*.

INTRODUCTION

Aphidophagous predators such as ladybird beetles are effective biological agents to control aphids in perennial and annual cultivated species. Introduction and conservation of beneficial insects are two ways to increase natural control of pests. The preservation of predator natural populations can be expected to ensure long term biological control of aphid pests in crop fields. Amongst the trapped ladybird species in vegetable crops, the ubiquitous *Propylea quatuorpunctata* was present but *Coccinella septempunctata* was the more abundant (Francis *et al.*, 2001). The large occurrence of the latter in fields was not surprising, this beetle species is mainly found in herbaceous habitats and is very polyphagous (Francis, 2001). However, the movement and dispersion of *C. septempunctata* and its ability to aphid predation over large areas is not really understood because of the difficulty in identifying the origins of predators.
In the present investigations we used a molecular technique employing polymerase chain reaction (PCR) to assay genetic variation in the total DNA extracted from *C. septempunctata*. RAPD(Randomly Amplified Polymorphic DNA)-PCR usually detects mostly nuclear DNA polymorphism. Differing RAPD patterns reflect differences in DNA sequences spread

through the genomes of organisms (Welsch and McClelland, 1990; Williams *et al.*, 1990).

MATERIALS AND METHODS

Sample collection

Ladybird specimens were collected from 12 locations in French Community of Belgium. Samples were then frozen at –20°C until use.

DNA isolation

To minimize microbial contaminations by non-ladybird DNA, DNA for RAPD-PCR analysis was isolated from head. DNA was extracted with QIAamp Blood and Tissue Kit (QIAGEN ®) according to the manual with the exception that in the final step DNA was resuspended in 100 μl TE-buffer.

RAPD amplification

RAPD-PCR amplifications were performed in a total volume of 25 μl containing 10mM Tris-HCl pH 8.3, 50 mM KCl, 1,5mM MgCl2, 100 μM dNTP, 10 pM primer, 1.5 U *Taq* polymerase (Amersham®) and 25 ng genomic DNA. Amplifications were carried out in a thermocycler (Eppendorf®, Master Cycler Gradient): first cycle 94°C for 5 min, 40°C for 2 min and 72°C for 3 min; then 39 cycles at 94°C for1 min, 40°C for 1.5min and 72°C for 2 min. PCR products were separated in 1.5% TAE agarose gels. Gels were run at 5 V/cm for 3 hours, stained with ethidium bromide and photographed under UV light. A search for polymorphism was performed from 16 decamer primers from Operon Technologies® generated more than hundred polymorphic RAPD bands (Figure 2). Two primers that revealed polymorphism were selected: OPBE-03 and OPBE-O9. A negative control (without DNA template) was added to every amplification run to ensure that scorable fragments were not artefacts. Pairwise distances were calculated between populations according to Nei and Li (1985), then used to construct a radial neighbour-joining dendrogram and examine intra- and inter-population variance coefficients, by analysis of molecular variation (AMOVA).

RESULTS AND DISCUSSION

To quantify the genetic diversity within the species and monitor the spatial foraging, 12 populations were sampled from Belgium (Figure 1) and analysed for RAPD DNA variation.

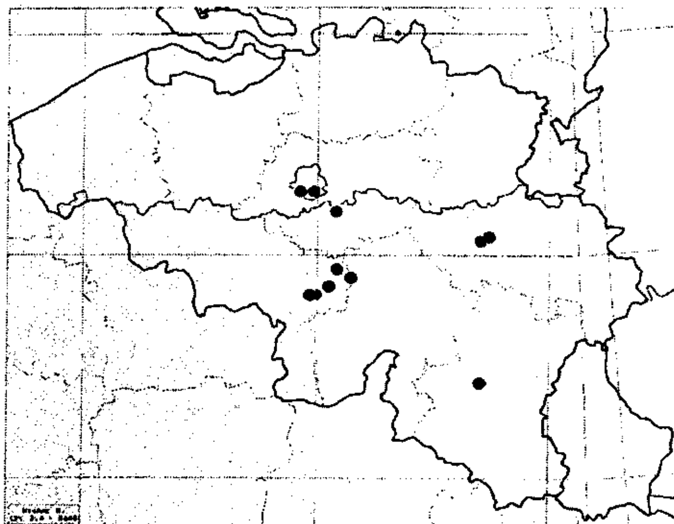

Figure 1: Geographic location of ladybird populations in Belgium

Performing the RAPD amplifications on the ladybird DNA, sixteen decamer primers from Operon Technologies generated more than hundred polymorphic RAPD bands (Figure 2).

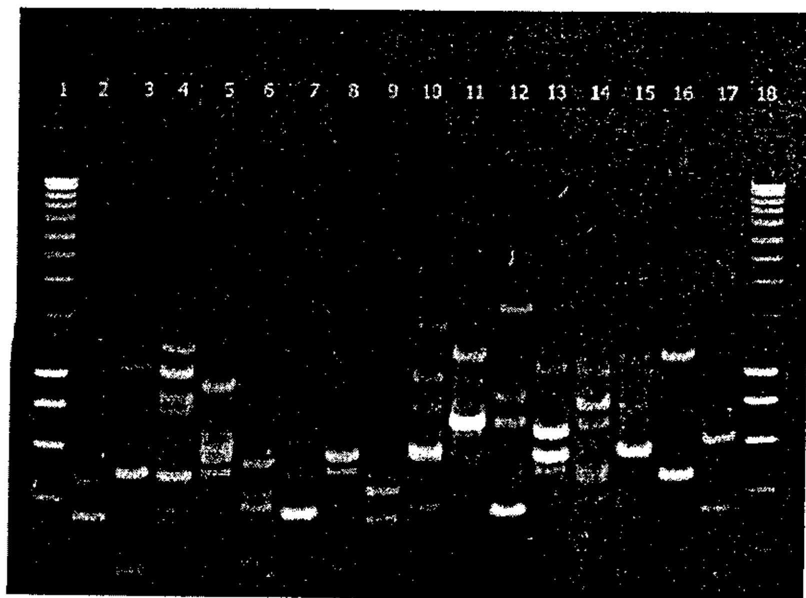

Figure 2: RADP patterns generated using sixteen 10-mer primers and DNA from *Coccinella septempunctata*. Lanes 1 and 18 = molecular marker ; lanes 2-17 = primers BE1-16 from Operon Technologies®.

A example of the dendrograms generated using the band frequencies for each populations is given in Figure 3. The present study demonstrated the usefulness of RAPD-PCR for differentiating populations of *C. septempunctata* with a high degree of confidence. These 12 populations could be correctly differentiated in five groups using two 10-mer primers.

Figure 3: Dendrogram of *Coccinella septempunctata* populations generated by UPGMA analysis using Nei and Li similarity based on shared fragments generated from primers OPBE-03 and OPBE-09.

Table 1: Corrected values of the Nei and Li coefficients of genetic similarity for DNA bands produced using primer BE3

Strains	SAM	GIL	MON	TAM	MSM	FLE	BOI	UCC	RIX	SHU	ENG	FLE
Sambreville	0.903	0.876	0.871	0.755	0.754	0.798	0.745	0.671	0.732	0.655	0.620	0.782
Gilly	-	0.935	0.788	0.833	0.777	0.722	0.718	0.788	0.765	0.812	0.655	0.623
Montigny-le-Tilleul	-	-	0.932	0.821	0.734	0.743	0.790	0.687	0.702	0.653	0.643	0.657
Tamines	-	-	-	0.887	0.786	0.775	0.811	0.641	0.800	0.699	0.671	0.679
Mont sur Machienne	-	-	-	-	0.876	0.778	0.654	0.722	0.623	0.687	0.592	0.735
Fleurus	-	-	-	-	-	0.987	0.697	0.634	0.698	0.641	0.597	0.782
Boisfort	-	-	-	-	-	-	0.896	0.651	0.712	0.598	0.6'72	0.690
Uccle	-	-	-	-	-	-	-	0.933	0.801	0.657	0.629	0.678
Rixensart	-	-	-	-	-	-	-	-	0.911	0.589	0.613	0.788
St Hubert	-	-	-	-	-	-	-	-	-	0.877	0.768	0.736
Engis	-	-	-	-	-	-	-	-	-	-	0.923	0.765
Lemal	-	-	-	-	-	-	-	-	-	-	-	0.966

The clustering of ladybird populations reflected the geographical distance separating the populations. The most genetically similar populations (1 to 6) originated from the same area (Hainaut), sharing between 72.2 to 87.6% of their positive bands. Populations 9 and 10 originated within 150 km of each other only shared 58.9% of their positive bands(Table 1). Although all of the ladybird populations exhibited a relatively high degree of genetic similarity with each other, a correlation between genetic similarity and the geographical distance among populations was observed ($r=-0.56$, $P=0.042$).

Before PCR technology availability, it was extremely difficult to differentiate individual ladybirds into population groups. Our results show that RADP-PCR technique seems to be a very useful molecular marker for maintaining predator introductions and tracking genes in the management of biological control. However, in our situation, the Nei and Li coefficient of genetic similarity and the cluster analysis may be an indicator of the difficulty to correctly differentiate the populations based on RAPD markers. The genetic diversity within sites might be influenced by population size in *C. septempunctata*; local population sizes are known to influence the degree of genetic variation within and between populations (Lande, 1995). Similar observations have been reported for *Plodia interpunctella* (Dowdy & McGaughey, 1996). However, for identifying predator populations originated as close as few kilometres the ones from the others, molecular methods such as microsatellites or SSR-PCR has to be used. All these molecular methodologies could be used as a basis to extend understanding of the ladybird ecology. Analysis of temporal and spatial variation should give indications for dispersion, foraging and genetic drift of ladybirds on microgeographic scale, lower than 1 km far.

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