The distribution and PCR-based fingerprints of *Rhyzopertha dominica* (F.) in Canada

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Abstract

The first record of *Rhyzopertha dominica* in North America is in 1861. Since then it has become a major pest in the USA and is found occasionally in Canada. We sought answers to the following questions: What is the extent of its distribution in Canada? What are the sources of infestation? What is its genetic background?

Pheromone traps detected *R. dominica* mainly in Manitoba, though it was trapped in the other western provinces. It was not caught in southern Ontario or Quebec. In Manitoba the total numbers caught from 9 traps were 2538 (1990), 4226 (1991), 723 (1992), and 289(1993). Despite extensive sampling, *R. dominica* was rarely detected in grain held in storage.

Genetic differences among *R. dominica* populations were investigated using the PCR-based fingerprinting method of Randomly Amplified Polymorphic DNA (RAPD-PCR). Surveyed genotypes from Canada were shared by many distantly separated populations in the USA, suggesting that gene flow and migration are widespread.

Introduction

Rhyzopertha dominica, the lesser grain borer (Coleoptera: Bostrichidae), is a major pest of stored cereals. It is thought to have originated from the Indian subcontinent, but now has a cosmopolitan distribution (Potter 1935). *R. dominica* was found in the USA in 1861 (Leconte 1862), with widespread distribution occurring by the 1920s (Back and Cotton 1922). *R. dominica* has been found in grain stored on farms in all USA states bordering the Canadian prairie provinces (Storey et al. 1983; Subramanyam and Harein 1989).

There are occasional records of *R. dominica* occurring in Canada since the late 1800s (Fields et al. 1993). However, several extensive surveys of insects in grain stored on farms in the Canadian prairies have failed to detect any *R. dominica* (Liscombe and Watters 1962; Loschiavo 1975; Smith and Barker 1987; Madrid et al. 1990). Each year the Canadian Grain Commission conducts Berlese funnel extractions for stored-grain insects in over 100000 samples of prairie grain from primary elevators (grain received directly from the farm), rail cars and terminal elevators (grain destined for export). From 1975 to 1993 only nine *R. dominica* were found, and all of these since 1989 (J. Van Loon, unpublished data).

Occasionally *R. dominica* has been found in feed mills (Steinbach, Manitoba 1981; Aldergrove, BC 1987; Lyster, Quebec 1987; P.G. Fields, unpublished data). As feed mills are heated throughout the winter and sometimes import feed-grade corn from the USA (Fields et al. 1993), we believe that corn infested with *R. dominica* from the USA could be a source of infestation for other stocks of grain in Canada. In 1990 over 600000 t of grain was imported from the USA into Canada; in 1991, less than 300000 t. In both years over 90% of the grain was corn. Assuming the infestation levels are similar to what they were in the late 1970s (Storey et al. 1982), then Canada would import over a million *R. dominica* each year (Fields et al. 1993).

The purpose of this study is to determine the distribution of this species across Canada and to determine genetic relationships among Canadian populations and between Canadian and U.S. populations. These data should be useful in testing three hypotheses: importation of infested grain, windborne migration (e.g. Smith and MacKay 1989) or an established Canadian population, put forth to explain the increased incidence of *R. dominica* in western Canada.

Materials and Methods

Pheromone trapping

Methods were similar to those of Fields et al. (1993). Eight-unit Lindgren funnel traps (Phero Tech Inc., Delta, BC, Canada) (Lindgren 1983; Cogburn et al. 1984; Leos-Martinez et al. 1986, 1987), were baited with *R. dominica* aggregation pheromone (Williams et al. 1981) (10 µL of dominicalure 1, 97% pure and 10 µL dominicalure 2, 95% pure) in 1990 and 1991 or with commercially available pheromone loaded onto rubber septa (Trécé Ltd., Salinas, USA) in 1992 and 1993.

In Manitoba, traps were placed within 5 m of the grain storage structures at three dairy farms, three primary elevators and three feed mills. Outside Manitoba, traps were placed near large grain terminals or transfer elevators. Each week the collection jars at the bottom of each trap were emptied and refilled with a solution (2 L water, 40 mL 6% sodium hypochlorite to slow microbial growth, 2 mL liquid soap to reduce the surface tension, and 500 mL ethylene glycol to reduce evaporation). In 1990 and 1991 pheromones were changed weekly, in 1992 and 1993 pheromones were changed every 4 weeks.

Daily maximum and minimum temperatures were collected from a Stevenson screen at Glenlea, Manitoba. For the summer of 1990, the temperatures at Glenlea were compared to 3 other locations in Manitoba (Altona, Plum Coulee, Niverville) closer to the other pheromone traps. No significant difference was found (ANOVA, $p=0.61$) between the four locations, therefore only one location was used.

In 1990, pheromone traps (eight-unit traps) were placed 100 and 1000 m north, south, east and west of two grain elevators located at Dominion City, Manitoba. Traps (four-unit traps) were also placed inside each elevator at the receiving door, loading to railcar door, the bottom of the bucket elevator and

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the top of the bucket elevator. Insects were collected weekly from 8 August to 10 October.

PCR fingerprinting

We used the polymerase chain reaction (PCR) to analyse randomly amplified polymorphic DNA (referred to as RAPD-PCR), to study genetic diversity within and among populations of *R. dominica*. RAPD-PCR was introduced by Williams et al. (1990) as a means of identifying large numbers of heritable genetic markers, and since has been applied to population studies (Hadrys et al. 1992). RAPD-PCR uses a single randomly designed 10-base primer that typically anneals to a number of complimentary sequences in the genomes of most organisms. Only a single primer is added to the PCR reaction, and products are amplified only when priming sites occur sufficiently close to one another (generally less than 2 kb) in an inverted orientation. RAPD products are heritable dominant genetic markers. Polymorphism in RAPD-PCR products, as evidenced by the presence or absence of a particular band, results from either a mutation in the sequence at one of the priming sites, or a change (duplication or deletion) in the intervening sequence yielding a fragment of different size.

Insects were collected using pheromone-baited multiple funnel traps with wheat in the collection jar. Live specimens were frozen at –80°C and held at –45°C until extraction. RAPD-PCR was conducted using techniques modified from Williams et al. 1990. Genomic DNA was extracted from frozen individual *R. dominica* by grinding in a Tris-HCl EDTA (disodium ethylene diamine tetraacetate) buffer containing SDS (sodium dodecyl sulfate). The preparation was incubated at 70°C for 30 minutes, proteins extracted with KOH, precipitated with isopropanol, washed in ethanol, dried, and resuspended in 10 µL water. The extract was diluted 10 fold again to yield enough material for over 100 PCR experiments. For PCR, 1 µL of dilute sample DNA, about 1–10 ng, was combined with 200 ng of a 10-base primer, 1.25 µL of Taq polymerase, '10x' buffer provided by the manufacturer for the Taq polymerase, 10 mM of $MgCl_2$ for DNA stability and 2 mM of dNTPs (a mix of adenine, guanine cytosine and thymine for primer extension). Total reaction volume in a sterile tube was 23 µL. An over-abundance of primer relative to sample DNA is important to ensure that primers have a high probability of annealing to available complimentary sites anywhere on the genome before genomic DNA reanneals in the cooling cycle. Multiple reaction tubes were set up in a thermal cycling machine. Our temperature program was 1) denaturation at 94°C for an initial 3 minutes; 2) annealing at 36°C; 3) extension at 72°C for 1 minute; 4) back to 94°C for 1 minute to begin a new cycle, for a total of 45 cycles. PCR products were separated by electrophoresis on a 3% agarose gel at 90 VAC for about 1.5 hours, and visualised under UV light following ethidium bromide staining. Frequency of RAPD products (bands on gels) were computed for each population.

Results and Discussion

Pheromone trapping

As in 1990 and 1991 (Fields et al. 1993), *R. dominica* was found across western Canada in 1992. Although over nine traps were placed in eastern Canada, no *R. dominica* were caught (Fig. 1). In 1993 the only *R. dominica* found outside of Manitoba were near grain terminals in Vancouver (Fig. 2).

There were no differences between the three site types, farms, elevators or feed mills in any of the four years (Table 1). This suggests that imported grain from the USA is not the sole source of the *R. dominica* found in Canada. Furthermore, southern Ontario and Quebec import grain from the USA, but no *R. dominica* were found in 1992 in these areas.

Intensive sampling around two elevators in southern Manitoba showed that the closer traps were placed to grain elevators the more *R. dominica* were caught (Fig. 3). However, eight traps placed inside the elevators caught only 41 insects, and all of these from the loading and receiving doors. We can think of two explanations for this. One, *R. dominica* are in the elevator but are rarely detected because they react much differently to pheromone baited traps in the confined space of an elevator. Two, the source of the *R. dominica* was not the elevators, and insects were trapped closer to the elevators because they were attracted to elevators because of the grain volatiles (Dowdy et al. 1993). This second explanation supports the windborne origin hypothesis.

Low temperatures may explain why fewer insects were caught in 1992 and 1993 than 1991 (Table 1, Fig. 4) No insects were caught when the mean daily maximum temperature for the week was below 18°C. There was an exponential increase in the number of *R. dominica* caught as the temperature increased (Fig. 4). It is doubtful that the change in pheromone delivery systems in 1992 would account for the reduction in *R. dominica* caught. We showed earlier that there was no difference in insects caught using the two systems (Fields et al. 1993).

PCR fingerprinting

Twenty populations of *R. dominica* from six geographic regions in North America are currently being studied, and data from eight populations are reported here. Ten different random primers were studied for utility in providing markers. To date, two gave consistently interpretable banding patterns

Table 1. The total number of *Rhyzopertha dominica* caught between late May and late August in pheromone baited flight traps outside various storage facilities in Manitoba Canada (n=3 per site type, mean ± standard error of the mean), and the monthly mean temperature for June, July and August from Glenlea, Manitoba.

	Site type[a]				
Year	Farms	Elevators	Feed mills	Totals[b]	Temperature (°C)
1990	458 ± 220	159 ± 68	229 ± 198	282 ± 100[ab]	19 ± 1
1991	403 ± 125	448 ± 237	521 ± 305	470 ± 119[a]	21 ± 1
1992	68 ± 41	94 ± 39	80 ± 77	80 ± 28[b]	15 ± 0.2
1993	42 ± 35	40 ± 21	14 ± 8	32 ± 13[b]	13 ± 1

[a]No significant differences between site types (ANOVA p=0.90).

[b]Significant differences between years are indicated by different letters (ANOVA p=0.006, Duncans MRT p<0.05).

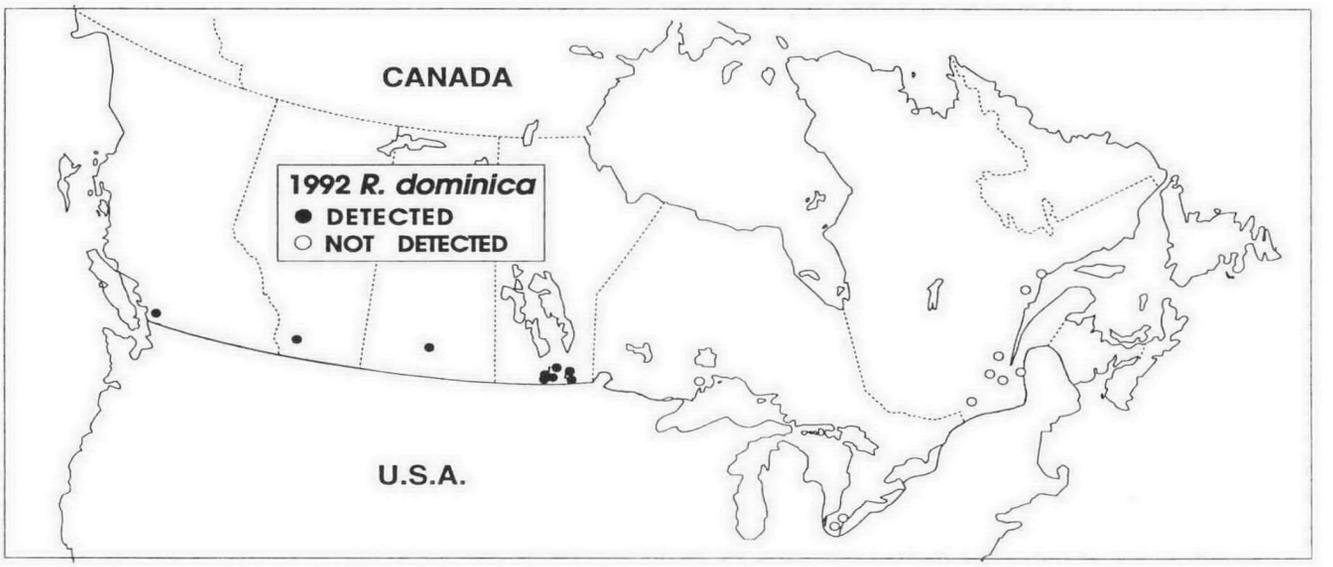

Fig. 1. The places that *Rhyzopertha dominica* were caught in pheromone-baited fight traps placed outside grain storage facilities in 1992: closed symbols had insects, open symbols did not.

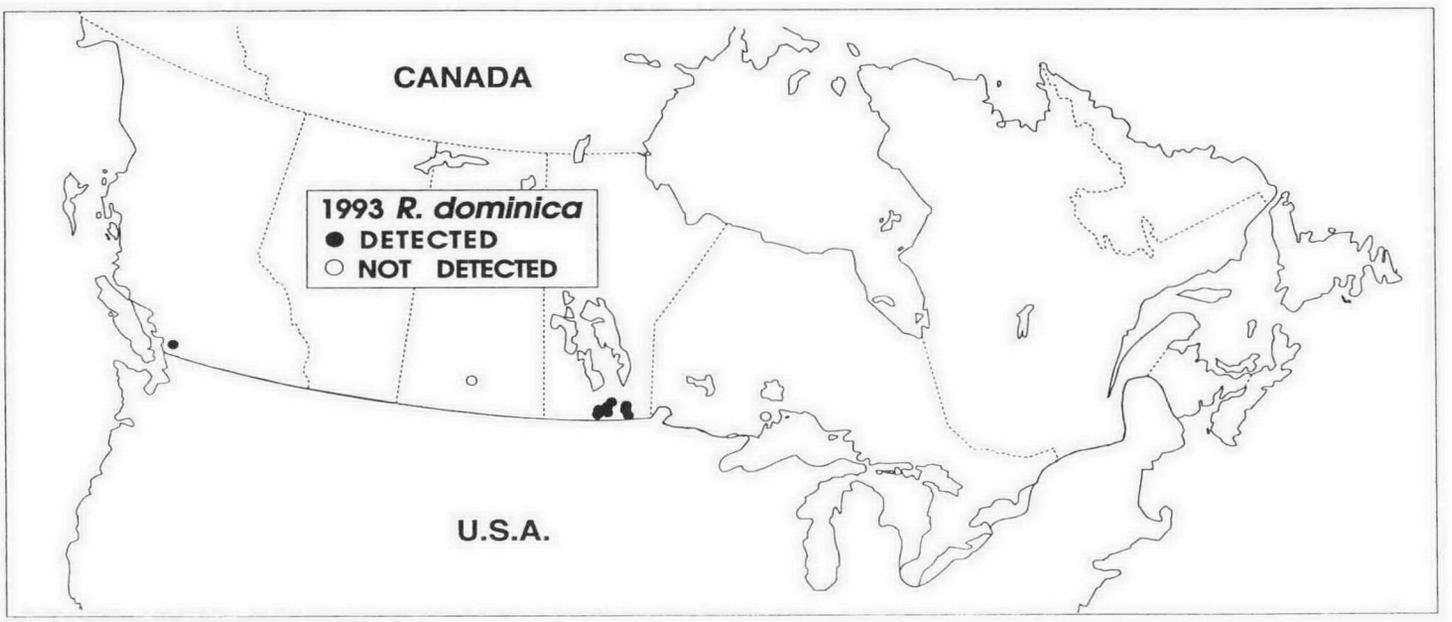

Fig. 2. The places that *Rhyzopertha dominica* were caught in pheromone-baited fight traps placed outside grain storage facilities in 1993: closed symbols had insects, open symbols did not.

(Fig. 5) and revealed polymorphism within and among populations. These two primers had the following sequences (5' to 3'): no. 160, CGATTCAGAG; no. 192, GCAAGTCACT.

Of the RAPD products observed following amplification with different primers, none were unique to any one *R. dominica* population and all occurred in each populatiosome level (Fig. 6). The only exception to this being the absence of 192/400 in the Coaldale Alberta population. Statistical analysis of the data is precluded by small sample sizes, but some relationships can be inferred at this time. Populations that differ substantially in band frequency are less closely related than those with similar band frequencies, and probably have not experienced gene flow recently. Populations fixed for presence or absence of a band possess less genetic diversity than those polymorphic at that band site (Hadrys et al. 1992). Thus our sample from Coaldale, Alberta is fixed for the presence of 160/700 and the absence of 192/400, which suggests that a genetic bottleneck occurred with the founding of this population by a few genetically similar individuals. If such a founder event was not followed by subsequent immigration of genetically variant individuals, then genetic diversity would be expected to remain low (Wright 1978). A recent founder event at the Coaldale site would be consistent with its status as the most remotely collected (far from commercial centres and established populations) *R. dominica* population in this study. More data on the frequency of genetic markers in *R. dominica* populations is needed to clarify genetic relationships among populations and discuss models of range expansion.

In summary, none of the three hypotheses concerning the origins of *R. dominica* were strongly supported or discounted. It is likely that grain imported from the USA contains some *R. dominica*, but we did not trap more *R. dominica* outside feed mills than other grain handling facilities. This strongly suggests that imported grain is not the sole source of insects. The windborne hypothesis is supported by the intensive

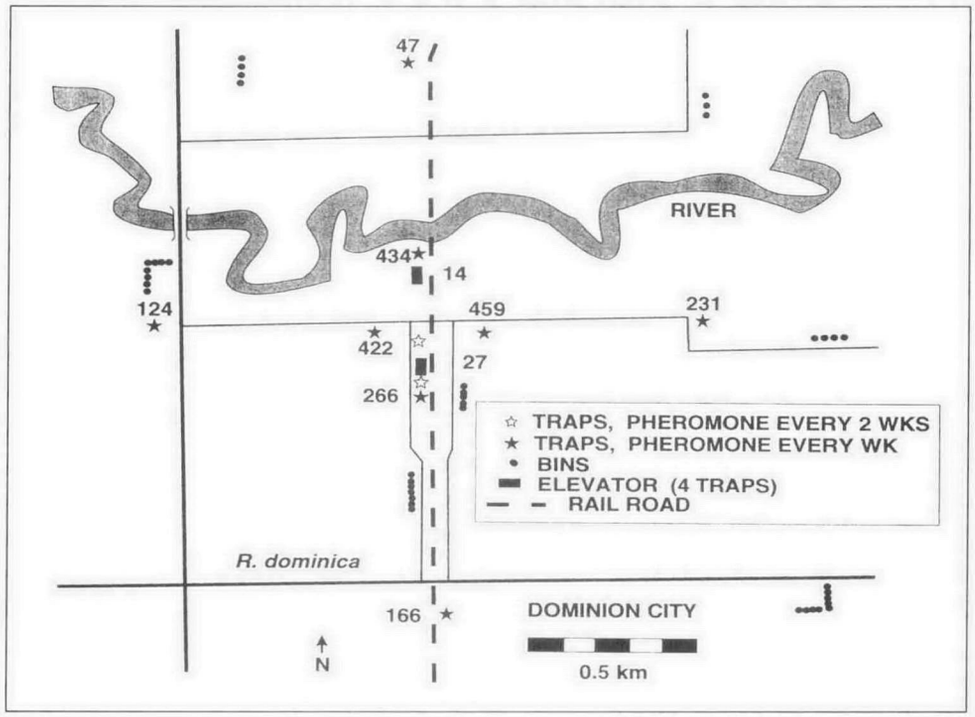

Fig. 3. The total number of *Rhyzopertha dominica* caught from 8 August to 10 October 1990 using pheromone-baited flight traps. There were four traps placed inside each of the two elevators.

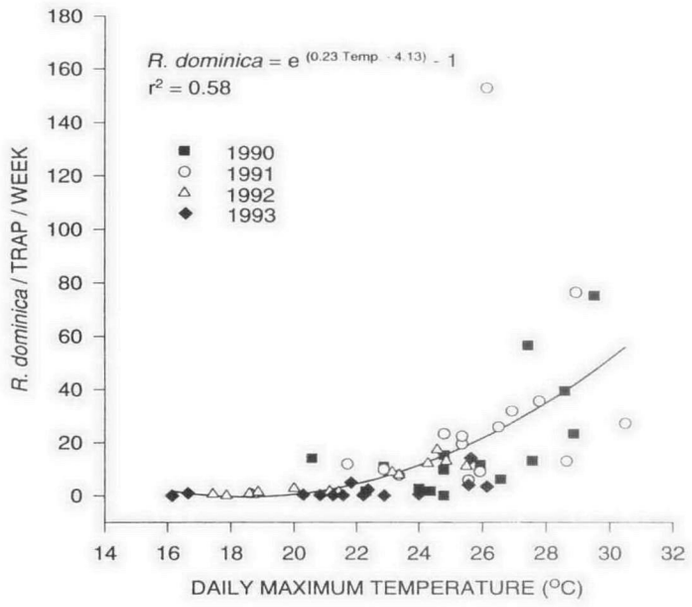

Fig. 4. The relationship between mean daily maximum air temperature at Glenlea Manitoba and the mean number of *Rhyzopertha dominica* caught in pheromone-baited flight traps located at farms, elevators and feed mills in Manitoba over one week.

sampling around elevators (Fig. 3). However, this evidence is circumstantial and further work would be required to prove it. Using RAPD-PCR, *R. dominica* from Canada share many genes with populations in the USA, suggesting the gene flow and migration are widespread. These data support the migration from the USA theory, though it does not distinguish the mode of transport. It suggests there are not locally established populations. Finally, preliminary experiments, not reported here, on the overwintering of *R. dominica* indicate that it cannot survive the winter in Manitoba. Despite large numbers of *R. dominica* caught in flight traps in western Canada over several years, this insect is still rarely found in stored grain.

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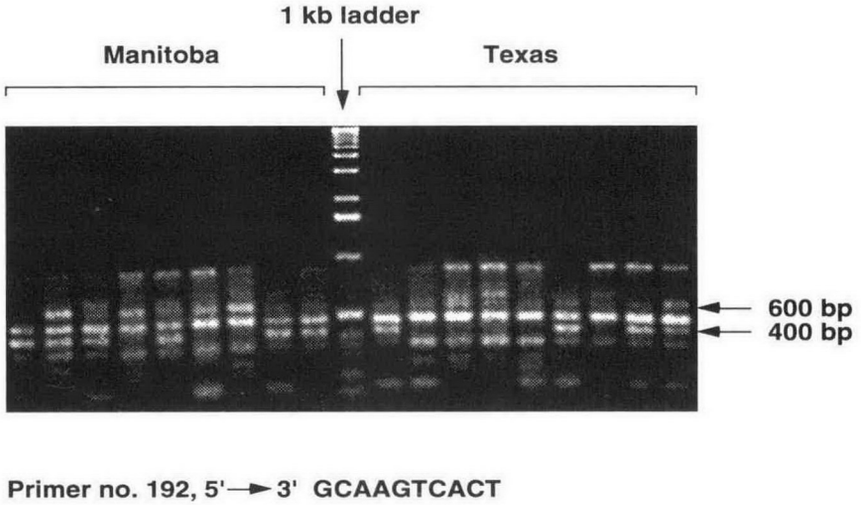

Fig. 5 A typical agarose gel showing RAPD products of *Rhyzopertha dominica* from two locations. The band at 500 base pairs is common to all individuals, while bands at 600 and 400 base pairs are polymorphic.

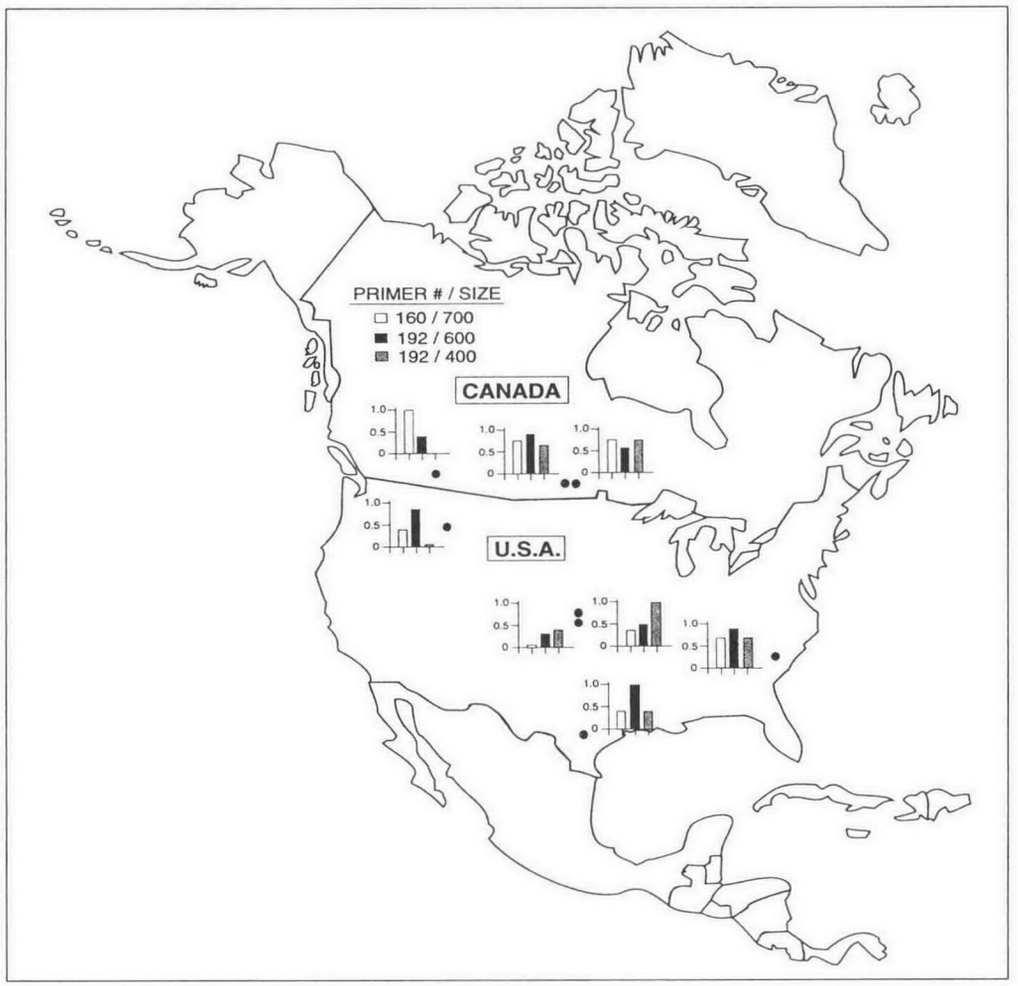

Fig. 6 The frequency of RAPD markers from *Rhyzopertha dominica* populations sampled across North America (n=8–11 per location).

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