

TECHNICAL NOTE

## *Mbo*I and *Macrohaltica* — quality of DNA fingerprints is strongly enzyme-dependent in an insect (Coleoptera)

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Originally developed as a forensic tool (Jeffreys, Wilson & Thein 1985), DNA fingerprinting has revolutionized the study of reproductive success in natural populations (Burke 1989). With its unprecedented precision in assigning parentage and identifying close relatives, the technique may well represent the most important methodological advance in behavioral ecology.

Multilocus DNA fingerprinting probes such as 33.15, 33.6 and M13 have been successfully applied to many vertebrate species (Bruford *et al.* 1992). By contrast, reports of success with arthropods have been slow in forthcoming (but see Carvalho *et al.* 1991; Achmann, Heller & Epplen 1992; Zeh *et al.* 1992). The difficulty in using these probes to obtain highly variable fingerprints from insects has impeded research into sexual selection. Many poorly understood phenomena, such as multi-male sperm competition and cryptic female choice (Eberhard 1990), could be best investigated through fingerprinting studies on insects.

Here, we compare fingerprints of an insect obtained using several probes and restriction enzymes. We demonstrate that, for 33.15 and 33.6, choice of enzyme has a critical effect both on clarity, i.e. band intensity relative to background, and on the level of band sharing. Two enzymes frequently used in vertebrate research consistently yielded the poorest results. Our findings may thus have general implications for DNA fingerprinting of insects.

Twenty-seven unrelated individuals of the chrysomelid, *Macrohaltica jamaicensis*, were collected near San José, Costa Rica. Beetles were frozen at  $-20^{\circ}\text{C}$  and stored in 100% ethanol. Fingerprinting procedures followed those in Zeh *et al.* (1992). Briefly, whole adults (*c.* 35 mg) were ground in 600  $\mu\text{l}$  of 2X CTAB extraction buffer, incubated with Proteinase K for 1.5 h at  $65^{\circ}\text{C}$ , and subjected to the following equal-volume extractions: one chloroform: isoamyl-alcohol (24:1); two phenol:chloroform:isoamyl-

alcohols (25:24:1) and a final chloroform:isoamyl-alcohol. DNA was precipitated in 67% ethanol, pelleted for 30 min at 13 000 r.p.m., and washed twice in 75% ethanol.

Samples were resuspended in 25  $\mu\text{l}$  of TE, digested for 6–8 h at  $37^{\circ}\text{C}$  with 40 units of *Hae*III, *Hinf*I or *Mbo*I, and run in 1.0% TBE agarose gels for 58 h at 36 V. DNA fragments were transferred to nylon membranes by capillary blotting (Bruford *et al.* 1992: protocol 2).

Three probes were utilized: 33.15, 33.6 and (CCAT)<sub>n</sub>. Plasmids containing 33.15 and 33.6 inserts (Cellmark Diagnostics) were transformed into *E. coli* JM101 (Sambrook, Fritsch & Maniatis 1989). Amplified plasmid DNA was isolated by alkaline lysis and inserts excised by *Eco*R1 and *Hind*III digestion. Probe inserts were isolated by electrophoresis in low-melting point gels (1.0%), purified by organic extractions, radiolabelled with [ $\alpha^{32}\text{P}$ ], and hybridized to beetle DNA (see Protocols 3 and 4 in Bruford *et al.* 1992). Synthesis, radiolabelling and hybridization of the probe (CCAT)<sub>n</sub> are detailed in May & Wetton (1991). Autoradiographs were scored using acetate overlays. Band-sharing coefficients (Wetton *et al.* 1987) were calculated by comparing individuals in adjacent lanes on a gel.

Ethanol preservation did not alter fingerprint patterns but reduced DNA yield by *c.* 50%. Inclusion of CTAB in the extraction protocol proved critical to complete digestion.

For 33.15 and 33.6, analyses of variance on arcsin-transformed data showed a highly significant effect of enzyme on band-sharing coefficient, *S* (33.15,  $F_{2,19} = 13.25$ ,  $P = 0.0003$ ; 33.6,  $F_{2,23} = 19.21$ ,  $P < 0.0001$ ). In both cases, *Mbo*I yielded significantly more variable fingerprints than *Hae*III or *Hinf*I (see Bonferroni 95% CI, Table 1). The difference between *Hae*III and *Hinf*I was not significant, although *Hinf*I fingerprints were least variable for both probes. For (CCAT)<sub>n</sub>, fingerprint variability was not significantly affected by enzyme ( $F_{2,21} = 1.62$ ,  $P = 0.2233$ ). Nonetheless, *Mbo*I fingerprints were again the most variable.

Enzyme effect on fingerprint clarity was even more striking (Fig. 1). With all three probes, *Mbo*I yielded high-definition fingerprints compared to the consistently lower band/background contrast produced by *Hae*III and *Hinf*I.

This study demonstrates that, given the correct choice of restriction enzyme, insect DNA fingerprints can rival

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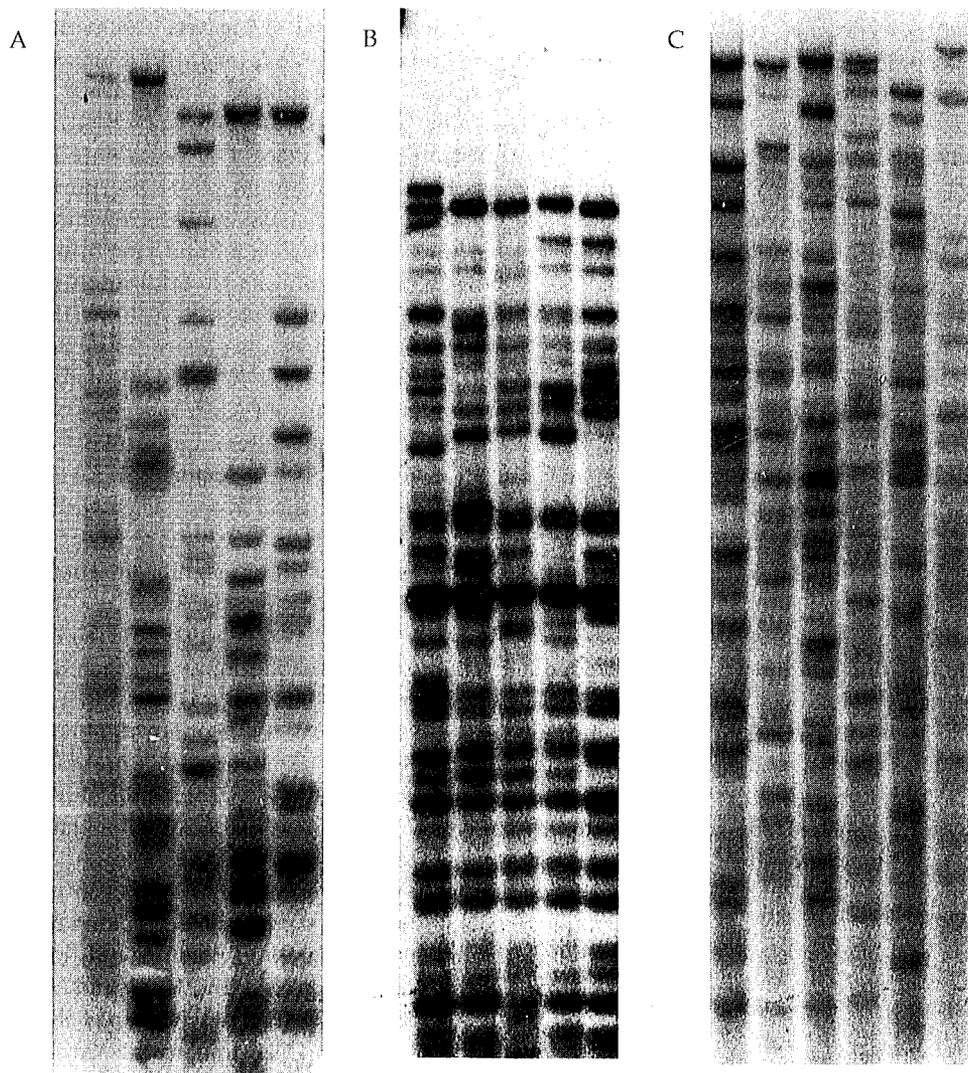
**Table 1** Mean band-sharing coefficient ( $S$ ), Bonferroni 95% confidence interval around  $S$ , and number of bands per individual ( $N$ ) categorized by probe and restriction enzyme

Enzyme	$S$	Bonferroni 95% CI	$N \pm SD$	$n$
Probe 33.15				
<i>MboI</i>	0.17	-0.02, 0.35	5.6 $\pm$ 1.6	7
<i>HaeIII</i>	0.59	0.42, 0.76	14.4 $\pm$ 3.1	8
<i>HinfI</i>	0.67	0.45, 0.88	22.0 $\pm$ 4.2	5
Probe 33.6				
<i>MboI</i>	0.18	0.07, 0.29	16.3 $\pm$ 3.5	10
<i>HaeIII</i>	0.53	0.41, 0.65	20.0 $\pm$ 3.6	9
<i>HinfI</i>	0.69	0.53, 0.85	21.4 $\pm$ 1.7	5
Probe CCAT				
<i>MboI</i>	0.19	0.09, 0.30	14.7 $\pm$ 4.6	9
<i>HaeIII</i>	0.19	0.05, 0.33	12.4 $\pm$ 2.7	5
<i>HinfI</i>	0.29	0.18, 0.40	10.4 $\pm$ 4.1	8

$n$  = number of profiles compared. SD = standard deviation.

human and avian fingerprints for clarity and levels of variability. Probed with both 33.6 and (CCAT)<sub>n</sub>, *MboI*-digested DNA yielded a mean of 31 bands ( $N$ ), with a band-sharing coefficient of 0.185. Although we have not yet performed inheritance studies to establish the true number of independently segregating bands, these data suggest a probability of profile sharing between unrelated *M. jamaicensis* ( $P$ ) of less than  $1 \times 10^{-20}$  ( $P \approx S^N$ ).

It seems significant that two of the most frequently utilized restriction enzymes yielded the poorest results. Because of its relatively high cost, use of *MboI* is generally restricted to single-locus DNA fingerprinting using charomid vectors (Bruford *et al.* 1992). Our original goal in testing *MboI* was, in fact, to produce gels for probing with both single- and multilocus probes. In the event, its consistently high performance across three multilocus probes suggests that investigators embarking on multilocus fingerprinting of insects should experiment with this little-used enzyme.



**Fig. 1** The good, the bad and the ugly of *Macrohalicta* fingerprints: (A) high variability, high-definition fingerprints with 33.6/*MboI*; (B) low variability with 33.6/*HinfI*; (C) moderate variability but high background with 33.6/*HaeIII*.

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