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Genetic Relationship and Allozyme Expression of Insecticide Susceptible and Resistant *Helopeltis theivora* Populations from Peninsular Malaysia

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ABSTRAK

Helopeltis theivora *dikenali sebagai perosak bagi koko dan teh di Malaysia. Beberapa mekanisme bagi kerintangan serangga telah dicadangkan, contohnya penurunan kadar sensitiviti bagi kawasan sasaran, detoksifikasi metabolik pestisid dan pengurangan kadar penembusan atau translokasi bagi racun serangga. Elektroforesis gel poliakrilamida (PAGE) digunakan untuk mengenal pasti enzim metabolik yang terlibat dalam perkembangan kerintangan bagi* Helopeltis theivora. *Serangga dewasa* Helopeltis theivora *diperoleh daripada tiga populasi yang berlainan iaitu Bukit Cheeding (Banting, Selangor), Sg. Palas (Cameron Highlands) dan MARDI (Cameron Highlands, Pahang). Dua puluh lima enzim telah diuji untuk menentukan polimorfisme dan 8 enzim telah dikenal pasti terdapat di dalam* Helopeltis theivora. *Dendogram yang dihasilkan daripada analisis kluster mengumpulkan populasi Banting dan MARDI dalam satu kumpulan manakala populasi Sg. Palas dikluster dalam kumpulan tersendiri.*

ABSTRACT

Helopeltis theivora *is a known pest of cocoa and tea in Malaysia. Several mechanisms of insecticide resistance have been proposed such as reduction in the sensitivity of target sites, metabolic detoxification of pesticides and decreased penetration or translocation of pesticides. Polyacrylamide gel electrophoresis (PAGE) was used to determine the metabolic enzymes involved in the development of resistance in* Helopeltis theivora. *Adults of* Helopeltis theivora *were collected from three geographical locations namely Bukit Cheeding (Banting, Selangor), Sg. Palas (Cameron Highlands) and MARDI (Cameron Highlands, Pahang). In total, 25 enzymes were screened for polymorphisms and 8 enzymes were detectable in* H. theivora. *The dendogram resulting from the cluster analysis grouped the infrequently sprayed Banting and the MARDI populations together while the intensively sprayed Sg. Palas population clustered by itself.*

INTRODUCTION

Helopeltis theivora is a known pest of cocoa (Entwistle 1972) and tea (Wilson 1999) in Malaysia. Insecticides have been used for the control of the mirids as cocoa is grown widely in this country. Dzolkhifli *et al.* (1998) reported the development of resistance in the Sungai Tekam, Pahang and Serdang populations of this insect to γHCH, deltamethrin and cypermethrin. Several mechanisms of insecticide resistance have been proposed such as reduction in the sensitivity of target sites, metabolic detoxification of pesticides and decreased penetration or translocation of pesticides. Among them, metabolic detoxification was shown to play a major role in insecticide resistance (Sun 1992). The metabolic enzymes involved are those with roles in oxidation (mixed function oxidase), reduction (gluthatione s- transferase) and hydrolysis (esterase) (Matsumura 1985). Organophosphate and carbamate resistances are associated with increased esterase activity in a variety of insects including aphids (Takada and Murakami 1988; Abdel-Aal *et al.* 1992), Colorado potato beetle (Anspaugh *et al.* 1995), cockroaches (Siegfried *et al.* 1990; Prabhakaran and Kamble 1993), and mosquitoes (Pasteur *et al.* 1984; Raymond *et al.* 1987). Insects use specific or

general esterases to accomplish detoxification and sequestration of these groups of insecticides and electrophoresis can play a major role in identifying the enzymes involved (Devonshire and Moore 1982). Based on allozyme electrophoresis, esterase was found to be involved in insecticide resistance in the fruit fly, *Drosophilla buzzati*, from two different populations in Australia (Barker 1994). Therefore the objective of this study was to determine the metabolic enzymes involved in the development of resistance in *H. theivora* by using polyacrylamide gel electrophoresis (PAGE).

MATERIALS AND METHODS

Adults of *Helopeltis theivora* were collected from three geographical locations namely Bukit Cheeding (Banting, Selangor), Sg. Palas (Cameron Highlands) and MARDI (Cameron Highlands, Pahang) (*Fig. 1*) in April 2002. The samples were kept at - 70°C and twenty individuals from each population were used for electrophoresis. Individuals of *H. theivora* were homogenized with 50ml distilled water. 7% polyacrylamide gel electrophoresis (PAGE) was carried out with an initial current of not more than 50mA at 230V for 3 hours until the bromophenol blue dye reached the anodal end of the gel. In total, 25 enzymes were screened for polymorphisms. The gels were stained for α-esterase (α-EST, EC 3.1.1.1) and β- esterase (β-EST, EC 3.1.1.2), octanol dehydrogenase (ODH, EC 1.1.1.37), acid phosphatase (ACP, EC 3.1.3.2), malate dehydrogenease (MDH, EC 1.1.1.37), sorbitol dehydrogenase (SDH, EC 1.1.1.14), glucose 6-phosphate dehydrogenase (G6PDH, EC 1.1.1.44), leucine aminopeptidase (LAP, EC 3.4.11.1), xanthine dehydrogenase (XDH, EC 1.2.1.37), alpha-glycererophosphate dehydrogenase (α-GPDH, EC 1.1.1.8), hexokinase (HK, EC 2.7.1.1), aldehyde oxidase (ALDOX, EC 1.2.3.1), phosphoglucose isomerase (PGI, EC 5.3.1.9), peptidase (PEP, EC 3.4.1.1), isocitrate dehydrogenase (IDH-NADP, EC 1.1.1.42), superoxide dismutase (SOD, EC 1.15.1.1), glutamate oxaloacetate transaminase(GOT, EC 2.6.1.1), acid phosphatase acid (ACP, EC 3.1.3.2), malic enzyme (ME, EC 1.1.1.40), alcohol dehydrogenase (ADH, EC 1.1.1.1), pyruvate kinase (PK, EC 2.7.1.40), lactate dehydrogenase (LDH, E.C 1.1.1.27), 3-Hydroxybutyrate dehydrogenase (HBDH, EC 3.1.1.31), alkaline phosphatase (AKP, EC 3.1.3.1) and adenylate kinase (AK, EC 2.7.4.3) activities separately by

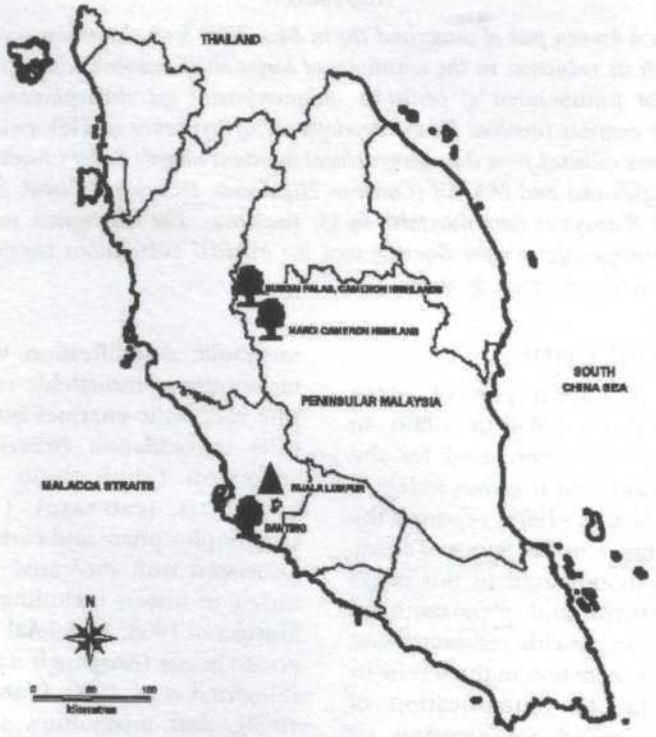

Fig. 1: Map showing sampling sites

the procedures of Shaw and Prasad (1970) with some modification. The POPGENE Version 1.31 computer package of Yeh and Boyle (1999) was used to calculate allelic frequencies, genetic distance (D) and F-statistics (Nei 1978). Based on the phenotypes observed for these eight enzymes in 20 samples from each of the three populations, alleleic frequencies were obtained which were in turn used to calculate the Nei's (1978) genetic distance coefficients among the three populations. The genetic distance coefficients were then used to cluster the populations by using UPGMA (unweighted pair group method with arithmetic averaging).

RESULTS AND DISCUSSION

The results showed that of the 25 enzymes screened, 8 enzymes were detectable in *H. theivora*. The 8 enzymes were α- and β- esterase (EST), xanthine dehydrogenase (XDH), leucine aminopeptidase (LAP), glutamate oxaloacetate transaminase (GOT), superoxide dismutase (SOD), glucose 6-phosphate dehydrogenase (G6PDH), aldehyde oxidase (ALDOX) and alkaline phosphatase (ALKP) (Table 1). Allelic frequency data for the 11 loci that could be scored in *H. theivora* are presented in Table 2, except for β-EST-2 that was monomorphic in all populations. The esterase loci showed different allelic frequencies in three populations. α-EST-1 showed a polymorphism in the Banting population. α-EST-2, and β-EST-1 were polymorphic in all populations, while α-EST-3 was monomorphic in all populations. XDH was polymorphic only in the Banting population but was monomorphic in the Sg. Palas and MARDI, Cameron Highlands populations. LAP and SOD were monomorphic in all populations while G6PDH and ALDOX were polymorphic in all populations. GOT was polymorphic in the Banting population but was monomorphic in the Sungai Palas and MARDI, Cameron Highlands population. AKP was polymorphic in the Banting and Sungai Palas, Cameron Highlands population but monomorphic in the MARDI, Cameron Highlands population. Allelic frequencies for the polymorphic loci ranged from 0.0625 to 0.9375 (Table 2). Values of *F*-statistics for *Helopeltis theivora* are presented in Table 3. The mean F_{is}, F_{it} and F_{st} values of 0.0493, 0.4822 and 0.4554 respectively, indicate the differentiation among the populations. The loci showing the highest F_{st} values had low values for gene flow (Table 3). The dendogram resulting from the cluster analysis (*Fig. 2*) grouped the Banting, Selangor and MARDI, Cameron Highlands populations together while the Sg. Palas, Cameron Highlands population clustered by itself. This clustering pattern could be due to the frequent spraying of insecticides at Sg. Palas, Cameron Highlands over the past 5 years (Philip Bauer, unpublished). For the Banting, Selangor and MARDI, Cameron Highlands populations, insecticides were not frequently sprayed when compared with the Sg. Palas, Cameron Highlands population. Based on the Nei's genetic distance value, the populations from Banting and MARDI, Cameron Highlands are closely related. In insects, esterases are known to be involved in important physiological processes, including the catabolism of juvenile hormone (Shanmugavelu *et al.* 2000), insecticide resistance (Prevec *et al.* 1992; Morton 1993; Mutero *et al.* 1994), digestion (Argentina and James 1995) and reproduction (Karotam and Oakeshott 1993). Insect esterase genes have shown high rates of intraspecific and interspecific

TABLE 1
Enzyme names, abbreviations, enzyme codes (E.C.), number of loci, enzymes structures and electrophoretic buffer systems

Enzyme	E.C. Number	Number of loci	Buffer system used
Alpha-esterase (α-EST)	3.1.1.1	3	Poulik
Beta-esterase (β-EST)	3.1.1.2	2	Poulik
Aldehyde oxidase (ALDOX)	1.2.3.1	1	Poulik
Superoxide dismutase (SOD)	1.15.1.1	1	Poulik
Glucose 6-phosphate dehydrogenase (G6PDH)	1.1.1.44	1	Poulik
Xanthine dehydrogenase (XDH)	1.2.1.37	1	TEB
Alkaline phosphatase (AKP)	3.1.3.1	1	Poulik
Glutamate oxaloacetate transaminase (GOT)	2.6.1.1	1	TEMM
Leucine aminopeptidase (LAP)	3.4.11.1	1	TEMM

TABLE 2
Allele frequencies and sample sizes (N) for 11 loci in *Helopeltis theivora* from three different populations

Locus	Allele	Banting	Sg. Palas, C.H	MARDI, C.H
α-EST-1	(N)	40	40	36
	110	0.5000	0.0000	0.0000
	100	0.5000	0.0000	0.0000
α-EST-2	(N)	40	40	22
	110	0.1000	0.2500	0.0000
	100	0.7000	0.7500	1.0000
α-EST-3	(N)	40	40	4
	10	0.0000	0.0000	0.5000
	100	1.0000	1.0000	0.5000
β-EST-1	(N)	40	32	40
	105	0.4000	0.2500	0.0000
	100	0.6000	0.7500	1.0000
XDH	(N)	40	40	10
	102	0.3000	1.0000	1.0000
	100	0.7000	0.0000	0.0000
LAP	(N)	14	38	10
	102	1.0000	0.0000	1.0000
	100	0.0000	1.0000	0.0000
GOT	(N)	40	40	36
	102	0.8750	0.3333	0.0000
	100	0.1250	0.6667	1.0000
SOD	(N)	32	12	18
	105	1.0000	0.1389	1.0000
	100	0.0000	0.8611	0.0000
	(N)	32	6	20
G6PDH	102	0.0625	1.0000	0.2000
	100	0.9375	0.0000	0.8000
ALDOX	(N)	40	30	32
	102	1.0000	0.2727	1.0000
	100	0.0000	0.7273	0.0000
	(N)	30	22	34
AKP	102	0.8667	0.2750	1.0000
	100	0.1333	0.7250	0.0000

*C.H= Cameron Highlands

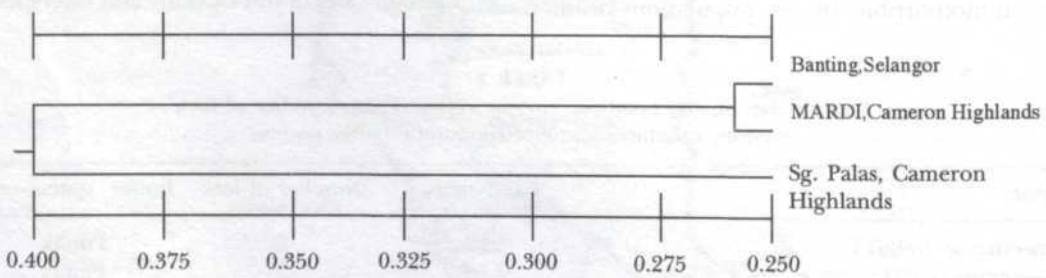

Fig. 2: Dendogram constructed by using UPGMA clustering of three populations of H. theivora *based on Nei's (1978) genetic distance coefficients*

TABLE 3
F-statistics values for the 12 loci of 3 populations of *Helopeltis theivora*

Locus	F_{is}	F_{it}	F_{st}	N_m
XDH	1.0000	1.0000	0.6087	0.1607
LAP	******	1.0000	1.0000	0.0000
GOT	-0.1429	0.7983	0.8235	0.0536
SOD	1.0000	1.0000	0.5714	0.1875
G6PDH	0.4045	0.4208	0.0273	8.9073
ALDOX	******	*****	1.0000	*****
AKP	0.5752	0.7827	0.4885	0.2617
_-EST-1	-0.8231	-0.7391	0.0460	5.1806
_-EST-2	0.6407	0.6825	0.1164	1.8977
_-EST-3	-1.0000	-0.2000	0.4000	0.3750
_-EST-1	-0.5205	-0.2766	0.1604	1.3087
_-EST-2	******	1.0000	1.0000	0.0000
Mean	0.0493	0.4822	0.4554	0.2990

*N_m =Gene flow estimated from $F_{st} = 0.25(1-F_{st})/F_{st}$

TABLE 4
Nei's Unbiased Measures of Genetic Identity (above diagonal) and Genetic Distance (below diagonal) (1978) for three different populations of *H. theivora*

Population	Banting	Sg.Palas, C.H	Mardi, C.H
Banting	******	0.6381	0.7732
Sg. Palas, C.H	0.4493	******	0.7097
Mardi, C.H	0.2573	0.3428	******

variation. Gene duplication followed by divergence of duplicated genes seem to be involved in the origin of at least part of this variability (Brady and Richmond 1992). The stability of insecticide resistance was found to be the result of modifications of the insect genome (Guillemaud *et al.* 1998; Raymond *et al.* 2001). In the sheep blowfly, *Lucilia cuprina*, there is a fitness cost associated with an allele coding for resistance to diazinon. But, following the intensive use of diazinon, a modifier gene had been selected in the field which completely supressed this cost. As a result, resistant insects carrying the modifier gene are equally fit as the susceptible insects.

CONCLUSION

Our results showed that the genetic structure of the population at Sg. Palas could have been affected by the intensive applications of insecticide when compared to the infrequently sprayed populations of Banting and MARDI, Cameron Highlands.

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