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Hexokinase, Malate Dehydrogenase, Fluorescent Esterase and Malic Enzyme Polymorphisms in the Cocoa Pod Borer, *Conopomorpha cramerella* (Snellen)

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Key words: *Conopomorpha cramerella;* **polymorphisms; hexokinase; malate dehydrogenase; fluorescent esterase; malic enzyme.**

ABSTRAK

Pengorek buah koko yang dikumpul dari ladang koko Tawau, Sabah dan Sua Betong, Negeri Sembilan, dan pengorek buah rambutan yang dikumpul dari Pucung dan kampus Universiti Pertanian Malaysia (UPM) di Serdang, Selangor, Malaysia telah dianalisis dengan menggunakan teknik elektroforesis gel poliakrilamid. Hexokinase didapati polimorfik pada populasi UPM, malat dehidrogenase pada populasi Tawau, Sua Betong dan UPM dan esteras fluorescent dan enzim malik adalah polimorfik pada ke empat-empat populasi.

ABSTRACT

Cocoa pod borers collected in the field from Tawau, Sabah and from Sua Betong, Negeri Sembilan and rambutan fruit borers collected from Puchong and the campus of Universiti Pertanian Malaysia (UPM) in Serdang, Selangor, Malaysia were analysed by polyacrylamide gel electrophoresis. Hexokinase was found to be polymorphic in the UPM population, malate dehydrogenase in the Tawau, Sua Betong and UPM populations and fluorescent esterase and malic enzyme were polymorphic in all four populations.

INTRODUCTION

The cocoa pod borer, *Conopomorpha cramerella*, is a major cocoa pest in Malaysia, Indonesia and the Philippines. In Malaysia two biotypes are present. The biotype in Sabah attacks both cocoa (*Theobromae cacao* L.) pods and rambutan (*Nephelium lappaceum* L.) fruits whereas the one in Peninsular Malaysia attacks only rambutan fruits but an outbreak of the biotype that attacks cocoa was reported for the first time in Peninsular Malaysia (in Malacca and Negeri Sembilan) towards the end of 1986 (Loke *et al.*, 1986, Ling *et al.*, 1987). Fortunately this outbreak in the peninsula has now been contained (Chin, 1987).

A review of the role that biochemical polymorphisms could play in the taxonomic study of this species had been presented elsewhere (Rita and Tan, 1987). We had previously reported on the occurrence of polymorphisms for the enzymes peptidase, α-glycerophosphate dehydrogenase (Rusnah *et al.*, 1985), phosphoglucomutase and esterase (Halmy *et al.*, 1987). In order to obtain meaningful data on the population genetics of this species, the number of polymorphic markers available for use to study this insect should be increased. We have therefore continued in our effort to find more biochemical polymorphisms in this species. We now report our findings that

the enzymes hexokinase (HK. E.C.2.7.1.1), fluorescent esterase (FE. E.C.3.1.1.1.), malate dehydrogenase (MDH. E.C.1.1.1.37) and malic enzyme (ME. E.C.1.1.1.40) are polymorphic when analysed electrophoretically in this economically important insect pest.

MATERIALS AND METHODS

C. cramerella was collected from cocoa pods of Sua Betong Estate near Port Dickson in Negeri Sembilan in November 1986 and from the Tawau District of Sabah, Malaysia in January 1987 based on the collection and transportation procedures described in Rusnah *et al.* (1985). *C. cramerella* from rambutans was collected from Ladang 7 in the campus of Universiti Pertanian Malaysia in Serdang in January 1987 and in Puchong, Selangor, Malaysia in September 1987 using the collection and rearing procedures of Rusnah *et al.*, 1985. The adult insects were stored at – 70°C until they were used for electrophoresis.

Sample homogenization and polycrylamide gel electrophoresis were done as in Rusnah *et al.* (1985) except for the following modifications. The CA-7 buffer system that was used to type for MDH had 0.1% Kodak Photoflo incorporated into the gel. We found that the addition of 0.1% Photoflo into our CA-7 gels improved the resolution of not only MDH but also esterase, phosphoglucomutase and α-glycerophosphate dehydrogenase bands. The concentration of Photoflo that we used was a reduction from the 1% used by Munstermann (1979) in his vertical gel system as that concentration unfortunately caused bubbling problems in the preparation of our horizontal gels. Use of 0.1% Photoflo solved the bubbling problem as well as improved the isozyme band resolution. HK, FE and ME were typed using the buffer system of Varvio Aho *et al.* (1980). Staining for MDH, HK and ME was as described in Stainer and Joslyn (1979) except that 0.1 M Tris-HCl, pH 8.0 was used as the staining buffer. Staining for FE was done following the procedure of Harris and Hopkinson (1976) for the enzyme carbonic anhydrase with fluorescein diacetate as the substrate. Testing for substrate specificity of FE was done by staining for general esterases with α- and β-napthyl acetates as substrates (Halmy *et al.*, 1987) and by staining for esterase D with 4 methylumbelliferyl acetate as the substrate (Harris and Hopkinson, 1976). Testing for inhibition by the carbonic anhydrase inhibitor acetazolamide, was done as described by Harris and Hopkinson (1976).

RESULTS AND DISCUSSION

A strong zone of HK activity was observed within 20 minutes of incubation. This zone migrated about 5 cm anodally from the origin when the bromophenol blue tracker dye migrated 13 cm anodally. On prolonged incubation of 1-2 hours, another faint zone of enzyme activity could be seen about 2 cm from the origin. However, as the occurrence of this faint zone was not a certainty it was therefore not scored. Three phenotypes were observed for the strong zone *(Fig. 1)*, phenotype HK 100/100 which showed a single slow band, 100/104 which showed a fast and a slow band and 104/104 which showed a single fast band.

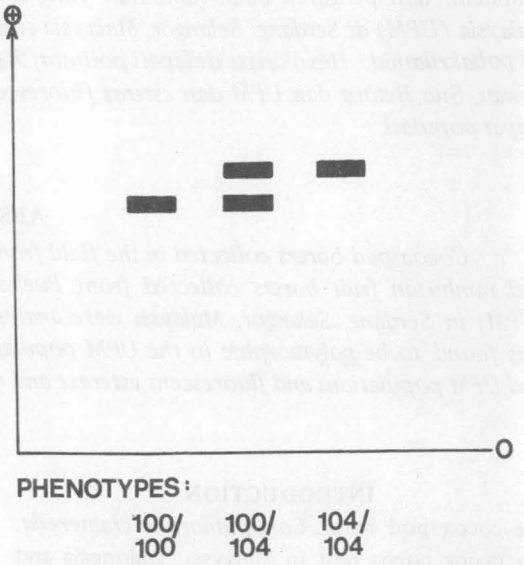

Fig. 1. Diagramatic representation of Hexokinase (HK) phenotypes observed in C. cramerella. *O is the origin.*

The population data for HK is presented in Table 1. Both males and females were about equally represented in all three phenotypes thus indicating that the HK electromorphs are coded for by an autosomal locus with two codominant alleles, HK^{100} and HK^{104}. For allelic designation,

TABLE 1
Population data for Hexokinase (HK) and Malate Dehydrogenase (MDH) in *Conopomorpha cramerella*. Numbers within brackets are expected numbers assuming Hardy-Weinberg equilibrium.

Enzymes & Populations	No. tested	Phenotypes 100/100	100/104	104/104	Gene Frequencies	
HK					HK^{100}	HK^{104}
Ladang 7	81	65 (64.00)	14 (16.00)	2 (1.00)	0.889 ± 0.025 $\chi_1^2 = 1.27$	0.111 ± 0.025 $P > 0.20$
MDH					MDH^{100}	MDH^{104}
Tawau	84	82 (82.01)	2 (1.96)	0 (0.01)	0.988 ± 0.008 $\chi_1^2 = 0.01$	0.012 ± 0.008 $P > 0.90$
Sua Betong	103	94 (94.20)	9 (8.61)	0 (0.20)	0.956 ± 0.014 $\chi_1^2 = 0.22$	0.044 ± 0.014 $P > 0.50$
Ladang 7	63	60 (60.04)	3 (2.93)	0 (0.04)	0.976 ± 0.013 $\chi_1^2 = 0.04$	0.024 ± 0.013 $P > 0.80$

we called the most common allele in the Ladang 7 population as allele "100". All other alleles were named based on the relative mobilities of their bands in mm to the band of allele "100". HK was only polymorphic in the Ladang 7 population which was in Hardy-Weinberg equilibrium for this enzyme. All 102 insects from Tawau, 112 insects from Sua Betong and 29 insects from Puchong showed phenotype 100/100 only.

Two zones of MDH were observed after 30 minutes of staining. The anodal MDH-1 migrated about 1 cm. The cathodal c-MDH migrated 0.2 em when the tracker dye migrated anodally 10 cm from the origin. c-MDH was monomorphic in all the four populations tested, while MDH-1 was polymorphic in the Tawau, Sua Betong and Ladang 7 populations. The phenotypes observed are shown in *Fig. 2.* Phenotype MDH-1 100/100 showed a single slow band while phenotype 100/104 showed three bands which is the typical pattern observed in the heterozygote for a dimeric protein such as MDH (Harris and Hopkinson, 1976). Both males and females are about equally distributed in the two phenotypes observed. We propose that the MDH-1 phenotypes are controlled by an autosomal locus with two codominant alleles $MDH\text{-}1^{100}$ and $MDH\text{-}1^{104}$. The population data for the three populations polymorphic for MDH-1 are presented in Table 1. All

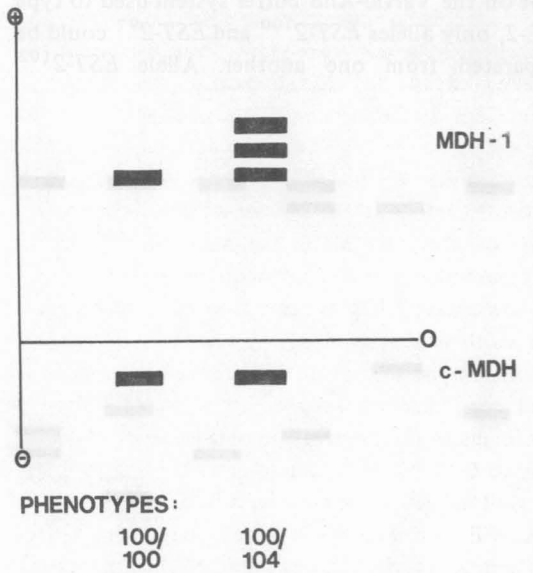

Fig. 2. Diagramatic representation of Malate Dehydrogenase-1 (MDH-1) phenotypes observed in C. cramerella. *The Cathodal c-MDH was monomorphic showing one band per sample. O is the origin.*

three populations are in Hardy Weinberg equilibrium for MDH-1. It is not surprising that we did not observe the predicted phenotype 104/104

with only one fast band present as its expected value in the all three populations was less than one. All 30 Puchong samples showed phenotype 100/100.

Three anodal zones of enzyme activity were observed when FE was stained for by using either fluorescein diacetate or 4-methyl umbellifery acetate as the substrate. FE-1 migrated about 1 cm, FE-2 about 2.5 cm and FE-3 about 5 cm when bromophenol blue migrated 13 cm from the origin. The banding patterns observed when using these substrates were identical but the bands were clearer and easier to score when fluorescein diacetate was used as the substrate. Hence this substrate was routinely used to type the insects and the gels were read after about 20 minutes of incubation. However, none of the enzyme zones were inhibited by acetazolamide, the carbonic anhydrase inhibitor. $\alpha-$ and $\beta-$ napthyl acetates stained only zone FE-3. This zone was found to be similar to EST-2 described by Halmy *et al.* (1987) but on the Varvio-Aho buffer system used to type FE-2, only alleles *EST-2*^{100} and *EST-2*^{97} could be separated from one another. Allele *EST-2*^{102} could not be typed on this buffer. Based on their abilities to utilize both 4-methylumbelliferyl acetate and fluorescein diacetate as substrates but not α- and β-napthyl acetates, and the fact that they are not inhibited by acetazolamide, it is likely that FE-1 and FE-2 are similar to human esterase D (Harris and Hopkinson, 1976). FE-1 was monomorphic and showed one band per sample in all the insects typed while FE-2 was polymorphic in all the four populations analysed. Eleven phenotypes had been observed for this enzyme *(Fig. 3)*. The homozygous phenotypes, FE-2 100/100, 105/105, 95/95 and 97/97 showed a single band each while the heterozygous phenotypes, 105/100, 100/95, 105/95, 105/97, 100/97, 100/90 and 95/97 showed two bands each, typical of a monomeric protein. The population data are presented in Table 2. Both males and females are about equally represented in the various phenotypes. Hence it is proposed that FE-2 is coded for by an autosomal locus with five autosomal codominant alleles *FE-2*^{100}, *FE-2*^{105}, *FE-2*^{97}, *FE-2*^{95} and *FE-2*^{90}. The rare allele, *FE-2*^{90}, and phenotype FE-2, 100/90, were not taken into account when

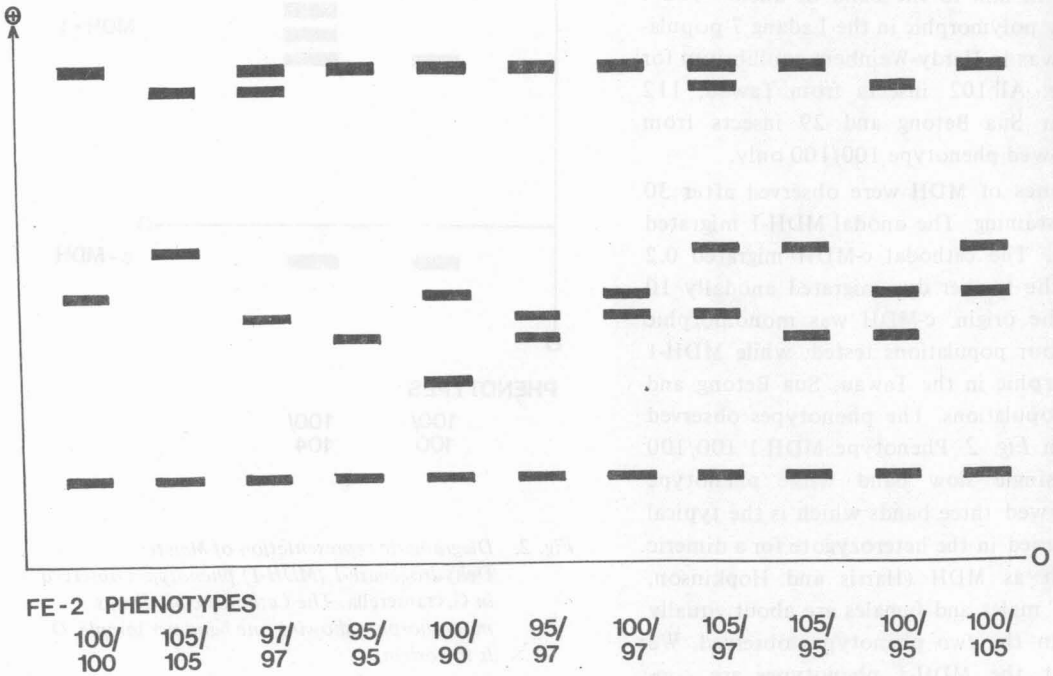

Fig. 3. Diagramatic representation of Fluorescent Esterase (FE) zones seen in C. cramerella. *FE-1 was monomorphic and FE-3 was not typed. All the FE-2 phenotypes observed are presented here. O is the origin.*

TABLE 2

Population data for Fluorescent Esterase – 2 (FE-2) in *Conopomorpha cramerella*. Numbers within brackets are expected numbers assuming Hardy-Weinberg equilibrium. N = number tested.

Phenotypes & Gene frequencies	Populations			
	Tawau	Sua Betong	Ladang 7	Pucung
100/100	25 (25.52)	16 (15.08)	10 (9.56)	6 (5.63)
105/105	1 (0.75)	5 (3.25)	1 (0.56)	2 (1.63)
97/97	0 (0.02)	0 (0.04)	0	1 (0.30)
95/95	1 (0.75)	3 (2.36)	1 (0.73)	2((1.41)
100/105	9 (8.75)	11 (13.98)	4 (4.61)	6 (6.07)
100/95	9 (8.75)	12 (11.91)	5 (5.27)	5 (5.63)
105/95	1 (1.50)	5 (5.54)	1 (1.27)	3 (3.03)
105/97	0 (0.25)	1 (0.72)	0	1(1.40)
100/97	2 (1.46)	2 (1.55)	0	3 (2.60)
97/95	0 (0.25)	0 (0.62)	0	1 (1.30)
100/90	0	1	0	1
N	48	56	22	30
FE-2^{100}	0.729 ± 0.045	0.518 ± 0.047	0.659 ± 0.072	0.433 ± 0.064
FE-2^{105}	0.125 ± 0.034	0.241 ± 0.040	0.159 ± 0.055	0.233 ± 0.055
FE-2^{97}	0.021 ± 0.015	0.027 ± 0.015	0	0.100 ± 0.039
FE-2^{95}	0.125 ± 0.034	0.205 ± 0.038	0.182 ± 0.058	0.217 ± 0.053
FE-2^{90}	0	0.009 ± 0.009	0	0.017 ± 0.017
	χ^2_6 = 1.09	χ^2_6 = 2.75	χ^2_6 = 0.630	χ^2_6 = 2.30
	P > 0.95	P > 0.80	P > 0.80	P > 0.80

the populations were tested for Hardy-Weinberg equilibrium. All four populations were in Hardy--Weinberg equilibrium thus supporting our genetic interpretation of this locus.

Two anodal zones of ME activity were observed after the gels were stained for about 30 minutes. The intensely stained ME-1 zone migrated about 1 cm while the weaker ME-2 zone migrated 2 cm when the tracker dye migrated 13 cm from the origin. ME-2 showed either two or three bands per sample but since it could not be scored with confidence it was not typed. Five phenotypes had been observed for ME-1 *(Fig. 4)*, phenotypes ME-1 100/100, 98/98 and 102/102 each showed a sharp single band while phenotypes 100/98 and 100/102 showed a broad band each. The broad band may represent five closely migrating bands since according to Harris and Hopkinson (1976), ME is a tetrameric protein. However, the molecular structure of this enzyme in *Conopomorpha* can only be confirmed through further biochemical studies which is beyond the scope of our expertise. All four populations are polymor-

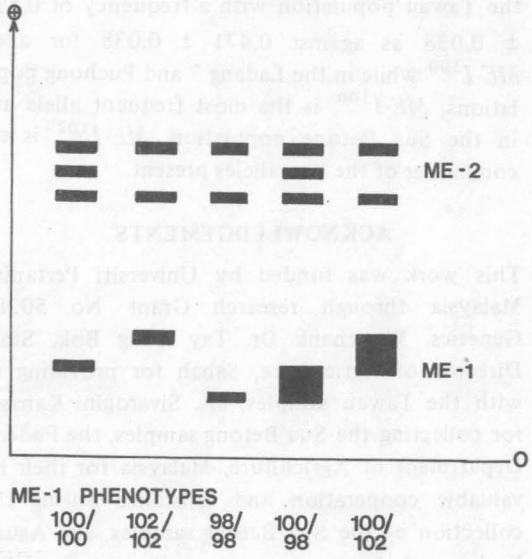

Fig. 4. Diagramatic representation of Malic Enzyme (ME) zones seen in C. cramerella. *ME-2 was not typed. All the ME-1 phenotypes observed are presented here. O is the origin.*

TABLE 3

Population data for Malic Enzyme-1 (ME-1) in *Conopomorpha cramerella*. Numbers within brackets are expected number assuming Hardy-Weinberg quilibrium. N = number tested.

Phenotypes & Gene frequencies	Populations			
	Tawau	Sua Betong	Ladang 7	Pucung
100/100	21 (18.82)	26 (24.52)	15 (14.77)	15 (14.63)
100/98	38 (42.35)	0	3 (1.85)	0
98/98	26 (23.82)	0	0 (0.06)	0
102/102	0	29 (27.52)	6 (4.67)	2 (1.63)
102/100	0	49 (51.96)	15 (16.62)	9 (9.75)
98/102	0	0	0 (1.04)	0
N	85	104	39	26
ME-1^{100}	0.471 ± 0.038	0.486 ± 0.035	0.615 ± 0.055	0.750 ± 0.060
ME-1^{98}	0.529 ± 0.038	0	0.038 ± 0.022	0
ME-1^{102}	0	0.514 ± 0.035	0.346 ± 0.054	0.250 ± 0.060
	$\chi^2_1 = 0.90$	$\chi^2_1 = 0.34$	$\chi^2_3 = 2.36$	$\chi^2_1 = 0.15$
	$P > 0.20$	$P > 0.50$	$P > 0.50$	$P > 0.50$

phic and in Hardy-Weinberg equilibrium for ME-1 (Table 3). Both sexes were present in about equal numbers in the phenotypes observed. We propose that ME-1 is controlled by an autosomal locus with three codominant alleles, *ME-1*^{100}, *ME-1*^{102} *and ME-1*^{98}. *ME-1*^{98} is the commoner allele in the Tawau population with a frequency of 0.529 ± 0.038 as against 0.471 ± 0.038 for allele *ME-1*^{100} while in the Ladang 7 and Puchong populations, *ME-1*^{100} is the most frequent allele and in the Sua Betong population, *ME-1*^{102} is the commoner of the two alleles present.

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