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An Electrophoretic Study of Natural Populations of the Cocoa Pod Borer, *Conopomorpha cramerella* (Snellen) from Malaysia.

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ABSTRAK

Pengorek buah koko dari Tawau, Sabah dan Sua Betong, Negeri Sembilan dan pengorek buah rambutan dari Serdang dan Puchong, Selangor dan Kuala Kangsar, Perak, Malaysia telah dianalisa secara elektroforesis dalam usaha untuk mendapatkan diagnosis elektromorf antar kedua biotip Conopomorpha cramerella. *30 enzim dan protein-protein umum telah dapat ditunjukkan pada zimogram-zimogram tetapi tidak ada satu pun yang boleh digunakan sebagai penanda diagnosis antara pengorek buah koko dengan pengorek buah rambutan. Frekwensi alil-alil untuk 8 enzim polimorf juga dipaparkan.*

ABSTRACT

Cocoa pod borers from Tawau, Sabah and Sua Betong, Negeri Sembilan and rambutan fruit borers from Serdang and Puchong, Selangor and Kuala Kangsar, Perak, Malaysia were subjected to electrophoretic analysis in an effort to find diagnostic electromorphs between these two biotypes of Conopomorpha cramerella. *Thirty enzymes and general proteins were successfully demonstrated on zymograms but none of them could serve as diagnostic markers between cocoa pod borers and rambutan fruit borers. The allelic frequencies for 8 polymorphic enzymes are presented.*

INTRODUCTION

The cocoa pod borer, *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracilariidae) is a major cocoa pest in Sabah State, Malaysia but until late 1986 it was only present as a minor pest of rambutan in Peninsular Malaysia (Loke *et al.* 1986, Ling *et al.* 1987). Both the cocoa pod borer and the rambutan fruit borer are known scientifically as *C. cramerella.* An outbreak of the cocoa biotype occurred for the first time in the states of Malacca and Negeri Sembilan in Peninsular Malaysia in September and November 1986 respectively. Fortunately the cocoa pod borer had not been detected elsewhere in the Peninsula (Chin 1987). This outbreak enabled us to collect both the biotype that attacks the pods of cocoa (*Theobromae cocoa* L.) and that which attacks rambutan (*Nephelium lappaceum* L.) fruits from the Peninsula within a period of three months (November 1986 to January 1987) although unfortunately not from the same locality. We were also able to obtain the cocoa biotype from Tawau, Sabah in January 1987. With these samples, we attempted to find diagnostic electormorphs between the two biotypes of *C. cramerella.*

MATERIALS AND METHODS

The following samples were available for electrophoretic analysis: cocoa pod borers from Sua Betong Estate (SB) near Port Dickson in Negeri Sembilan collected in November 1986 and from the Tawau district of Sabah collected in January

1987 (T1); rambutan fruit borers from Ladang 7 in the campus of Universiti Pertanian Malaysia, Serdang, Selangor collected in January 1987 (L1); from Kuala Kangsar, Perak State (KK) collected in January 1987 and from Puchong, Selangor state collected in January 1987 (P1) and in September 1987 (P2). The adult insects were frozen at – 70°C until they were used for electrophoresis.

Sample homogenization and polyacrylamide gel electrophoresis were done as in Rusnah *et al.* (1985). Various buffer systems such as TEB (Green 1977), CA-7 (Steiner and Joslyn 1979), TEMM (Spencer *et al* 1964) and that of Varvio-Aho *et al.* (1980) were used in our screening for diagnostic electromorphs between cocoa pod borers and rambutan fruit borers. The staining procedures used were from Steiner and Joslyn (1979), Harris and Hopkinson (1976), Menken (1980), Shaw and Prasad (1970) and Munstermann (1979). Isoelectric focusing was performed as in Tan *et al.* (1982) using LKB ampholytes with pH ranges of 5-7 and 4-6 after which general protein were stained for by using Coomassie Brilliant Blue R250.

The polymorphic markers, phosphoglucomutase (PGM), esterase (EST) (Halmy *et al.* 1987), α-glycerophosphate dehydrogenase (α-GPDH), peptidase (PEP) (Rusnah *et al* 1985) and malate dehydrogenase were typed on CA-7 buffer with 0.1% Kodak Photoflo incorporated into the gel while hexokinase (HK), fluorescent esterase (FE) and malic enzyme (ME) were typed on the Varvio-Aho buffer (Tan *et al* 1987). PEP was also typed on the Varvio-Aho buffer.

RESULTS AND DISCUSSION

At least two adult insects each of cocoa pod borers from Tawau (T1) and Sua Betong (SB) and at least two adult insects each of rambutan borers from Ladang 7 (L1) and Puchong (P1 or P2) were screened for the presence of diagnostic electromorphs of the following enzymes that were successfully demonstrated on zymograms: aldehyde dehydrogenase, aldehyde oxidase, trehalase, glucose dehydrogenase, aconitase, alcohol dehydrogenase, fumarase, phosphoglucose isomerase, xanthine dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, diaphorase, fluorescent acid phosphatase, fucose dehydrogenase, L-threonine dehydrogenase, succinic dehydrogenase, uridine monophosphate kinase, glutamate oxaloacetate transminase, fructose 1, 6 diphosphatase, pyruvate kinase and acid phosphatase.

However, no consistent diagnostic electromorphs were observed between rambutan fruit borers and cocoa pod borer for any of the above enzymes. No diagnostic protein bands were observed between cocoa pod borers and rambutan fruit borers although numerous bands were seen on the gel for each insect sample after isoelectric focusing runs on pH ranges 4-6 and 5-7. The allelic frequencies for the polymorphic systems are presented in Table 1. None of these polymorphic systems showed any diagnostic electromorphs between CPB and RB. The data for populations T2 (CPB from Tawau, collected in December 1984) and P3 (RB from Puchong, collected in January 1985) are from Rusnah *et al.* (1985) and that for L2 (RB from Ladang 7 UPM collected in March-April 1985) is from Halmy *et al.* (1987). The data for HK, MDH, FE-2 and ME-1 for populations T1, SB, P2 and L1 are obtained from Tan *et al.* (1987) which reported in detail the occurrence of polymorphisms for these enzymes in *C. cramerella*, their biochemical characteristics and their proposed modes of genetic control. All the allelic frequencies from these papers are compiled in Table 1 together with data reported here for the first time for comparative purposes and in order to calculate their heterozygosity values. Data for EST-1 is not presented here as it could not be reliably typed in this study because the quality of the EST-1 bands deteriorated on storage during the time span of about ten months from the time the samples were first collected to the time the electrophoretic analysis was finally completed. Fortunately, the bands for the other polymorphic systems remained consistent and could be typed reliably during the study period.

The addition of Kodak Photoflo to the CA-7 gel improved the resolution of the PGM bands so that another allele, PGM^{98} which was not detected previously (Halmy *et al.* 1987) could now be typed. PEP was initially typed on

TABLE 1

Gene frequency data for Esterase-2 (EST-2), phosphoglucomutase (PGM), peptidase-2 (PEP-2), α–glycerophosphate dehydrogenase (α-GPDH) hexokinase (HK), malate dehydrogenase (MDH), fluorescent esterase -2 (FE-2) and malic enzyme (ME-1) in natural populations of *Conopomorpha cramerella* (Snellen) from five localities in Malaysia.

	Allelic frequencies								
Locus & Allele	*T1*	*T2*	*SB*	*L1*	*L2*	*P1*	*P2*	*P3*	*KK*
PGM									
105	0.231 ± 0.029	NA	0.344 ± 0.033	0.352 ± 0.038	0.302 ± 0.032	0.340 ± 0.067	0.500 ± 0.083	NA	0.304 ± 0.068
100	0.639 ± 0.033		0.543 ± 0.034	0.512 ± 0.039	0.476 ± 0.034	0.420 ± 0.070	0.361 ± 0.080		0.522 ± 0.074
98	0.038 ± 0.013		0.033 ± 0.012	0.049 ± 0.017	–	0.080 ± 0.038	0		0.044 ± 0.031
97	0.087 ± 0.020		0.080 ± 0.019	0.086 ± 0.022	0.221 ± 0.028	0.160 ± 0.052	0.140 ± 0.058		0.130 ± 0.050
N	104*		106*	81*	106*	25*	18*		23*
H	0.538		0.591	0.593	0.642	0.640	0.611		0.565
EST-2									
102	0.279 ± 0.031	NA	0.361 ± 0.031	0.375 ± 0.039	0.355 ± 0.037	0.406 ± 0.087	0.375 ± 0.070	NA	0.417 ± 0.082
100	0.553 ± 0.035		0.574 ± 0.032	0.487 ± 0.041	0.428 ± 0.038	0.469 ± 0.088	0.479 ± 0.072		0.389 ± 0.081
97	0.168 ± 0.026		0.066 ± 0.016	0.138 ± 0.028	0.217 ± 0.032	0.125 ± 0.054	0.146 ± 0.051		0.194 ± 0.067
N	104*		122*	76*	83*	16*	24*		18*
H	0.567		0.525	0.592	0.614	0.563	0.708		0.444
PEP-2 (CA-7 buffer)									
F	0.947 ± 0.036	0.919 ± 0.022	1.000	1.000	NA	1.000	NA	1.000	1.000
S	0.053 ± 0.036	0.081 ± 0.022	0	0		0		0	0
N	19*	74	18*		19*	18*		41*	14*
H	0.105	0.162	0	0		0		0	0
	0								
HK									
104	0	NA	0	0.111 ± 0.025	NA	0.042 ± 0.041	0	NA	0.080 ± 0.038
100	1.000		1.000	0.889 ± 0.025		0.958 ± 0.041	1.000		0.920 ± 0.038
N	102		112	81		12*	28		25*
H	0		0	0.173		0.083	0		0.080
PEP-2-(Vario-Aho's buffer)									
102	0.144 ± 0.037	NA	0	0	NA	NA	0	NA	NA
100	0.800 ± 0.042		0.932 ± 0.027	0.895 ± 0.050			0.940 ± 0.034		

98	0.056 ± 0.024		0.068 ± 0.027	0.105 ± 0.050			0.060 ± 0.034			
N	45*		44*	19*			25*			
H	0.311		0.136	0.211			0.12			
α–GPDH										
F = 100	0.995 ± 0.005	1.000	0.996 ± 0.004	0.956 ± 0.019	NA	1.000	0.981 ± 0.019	0.989 ± 0.005	0.917 ± 0.046	
S = 97	0.005 ± 0.005	0	0.004 ± 0.004	0.018 ± 0.012		0	0.019 ± 0.019	0.011 ± 0.005	0.083 ± 0.046	
104	0	0	0	0.026 ± 0.015		0	0	0	0	
N	98*	86	118*	57*		18*	26*	184	18*	
H	0.010	0	0.008	0.088		0	0.038	0.022	0.167	
MDH										
104	0.012 ± 0.008	NA	0.044 ± 0.014	0.024 ± 0.013	NA	0	0	NA	0	
100	0.988 ± 0.008		0.956 ± 0.014	0.976 ± 0.013		1.000	1.000		1.000	
N	84		103	63		5*	24		20*	
H	0.024		0.087	0.048		0	0		0	
FE-2										
105	0.125 ± 0.034	NA	0.241 ± 0.040	0.159 ± 0.055	NA	NA	0.233 ± 0.055	NA	NA	
100	0.729 ± 0.045		0.518 ± 0.047	0.659 ± 0.072			0.433 ± 0.064			
97	0.021 ± 0.015		0.027 ± 0.015	0			0.100 ± 0.039			
95	0.125 ± 0.034		0.205 ± 0.038	0.182 ± 0.058			0.217 ± 0.053			
90	0		0.009 ± 0.009	0			0.017 ± 0.017			
N	48		56	22			30			
H	0.438		0.554	0.455			0.633			
ME-1										
102	0	NA	0.514 ± 0.035	0.346 ± 0.054	NA	NA	0.250 ± 0.060	NA	0.304 ± 0.068	
100	0.471 ± 0.038		0.486 ± 0.035	0.615 ± 0.055			0.750 ± 0.060		0.696 ± 0.068	
98	0.529 ± 0.038		0	0.038 ± 0.022			0		0	
N	85		104	39			26		23*	
H	0.447		0.471	0.462			0.346		0.435	
$\bar{H}$	0.280	0.075	0.285	0.322	0.630	0.277	0.311	0.0178	0.255	
H1	0.240	–	0.244	0.321	–	0.342	0.242	–	0.250	

T1 = Tawau 1987, T2 = Tawau 1984, SB = Sua Betong 1986, L1 = Ladang 7 1987, L2 = Ladang 7 1985, P1 = Puchong Jan., 87, P2 = Puchong Sept., 87, P3 = Puchong 1985, KK = Kuala Kangsar 1987, N = sample size, H= Heterozygosity, $\bar{H}$ = Mean Heterozygosity, H1 = Mean Heterozygosity based on PGM, EST-2, HK, α-GPDH & MDH only. NA = Not analysed. * = Data presented for the first time in this paper.

the CA-7 buffer system used by Rusnah *et al.* (1985) but it was subsequently typed on the Varvio-Aho buffer because on this buffer three alleles could be typed for *PEP*-2 whereas on the CA-7 buffer, only two alleles could be recognised.

PGM, EST-2, PEP-2 (Varvio-Aho buffer), FE-2 and ME-1 were polymorphic in all the populations that were analysed for these enzymes. Slight differences were observed in the allelic frequencies between the various geographical populations and between populations from the same locality but collected at different times. However, there were no clear cut differences between the allelic frequencies and heterozygosities for populations of rambutan fruit borers and cocoa pod borers.

PEP-2 on CA-7 buffer showed polymorphism only in the Tawau sample of cocoa pod borers where alleles *PEP-2*^{F} and *PEP-2*^{S} (Rusnah *et al.* 1985) were present whereas in all the other populations typed only allele *PEP-2*^{F} was present. On the Varvio-Aho buffer, allele *PEP-2*^{102} was only present in the Tawau population whereas alleles *PEP-2*^{100} and *PEP-2*^{98} were present in the Tawau and Sua Betong cocoa pod borer populations and in the Ladang 7 and Puchong populations of rambutan fruit borers. Hexokinase was only polymorphic in the Ladang 7, Puchong and Kuala Kangsar populations of rambutan fruit borers in which both alleles *HK*^{104} and *HK*^{100} were present but the most common allele in these populations, namely *HK*^{100}, was fixed in the cocoa pod borer populations of Tawau and Sua Betong. α-GPDH was polymorphic in the Tawau, Sua Betong, Ladang 7, Puchong and Kuala Kangsar populations with alleles α-*GPDH*^{100} (α-*GPDH*^{F} in the terminology of Rusnah *et al.* 1985) and α-*GPDH*^{97} (or -*GPDH*^{S}) being present but allele α-*GPDH*^{104} was only present at a low frequency in the Ladang 7 population. MDH was only polymorphic in the cocoa pod borer populations of Tawau and Sua Betong and in the rambutan fruit borer population of Ladang 7 where allele *MDH*^{104} occurred at low frequencies. The Puchong and Kuala Kangsar populations were fixed for the common *MDH*^{100} allele. As regards ME-1, *ME-*^{98} was the commoner allele in the cocoa pod borer populations of Tawau, *ME*-1 ^{102} in the cocoa pod borer population of Sua Betong while the most frequent allele in the rambutan fruit borer populations from Ladang 7, Puchong and Kuala Kangsar was *ME*-1 ^{100}.

Hence, while differences do exist in allelic frequencies between populations of *C. cramerella* from different geographical areas of Malaysia, such differences also exist between populations from the same locality but collected at different times. Unique alleles were present in certain populations but the commoner alleles were present in all populations. We have therefore been unable to find any diagnostic gene or allele or even to build up a biochemical key that would enable us to distinguish electrophoretically between cocoa pod borers and rambutan fruit borers in our present study using electrophoresis.

H, the average heterozygosity value, calculated based on data for PGM, EST-2, HK, α-GPDH and MDH which are available for T1, SB, L1, P1, P2 and KK showed values in the range of 0.240 to 0.342 with no consistent differences between the two populations of cocoa pod borers and the four populations of rambutan fruit borers. (P1 & P2 differed in their times of collection from the same locality).

Rusnah *et al.* (1985) had suggested the possibility that a large scale electrophoretic survey of *C. cramerella* from Sabah and Peninsular Malaysia may reveal the existence of two species. However, in this study we were unable to find any evidence to support this suggestion. Further work using more powerful biochemical genetic tools like restriction fragment length polymorphisms (RFLP) should be done to determine whether diagnostic biochemical markers really do not exist between cocoa pod borers and rambutan fruit borers. However, financial constraints have prevented us from using these techniques.

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