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Allozyme variation and an estimate of the inbreeding coefficient in the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae)

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Abstract

Seven presumed Mendelian enzyme loci (*Est*-2, *Est*-3, *Gpi*, *Idh*-1, *Idh*-2, *Mdh*-2 and *Mpi*) were characterized and tested for polymorphism in coffee berry borers, *Hypothenemus hampei* (Ferrari), sampled in Côte d'Ivoire, Mexico and New Caledonia. The average genetic diversity was $H=0.080$. Two loci, *Mdh*-2 and *Mpi* were polymorphic, and thus usable as genetic markers. The population structure of *H. hampei* was analysed using Weir & Cockerham's estimators of Wright's *F*-statistics. A high degree of inbreeding ($f=0.298$) characterized the elementary geographic sampling unit, the coffee field. The estimate of gene flow between fields within a country was $Nm=10.6$ and that between countries was $Nm=2$. The population genetic structure in *H. hampei* could be related to its known population biological features and history.

Introduction

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), is the most serious insect pest of coffee, present in most of the coffee-producing regions of the world (Ticheler, 1961; Le Pelley, 1968; Giordanengo, 1992). In New Caledonia, insecticide control of *H. hampei* has recently failed because this species has developed high levels of resistance to cyclodiene insecticides (Brun *et al.*, 1989). A better knowledge of the reproductive biology and of the population genetics of *H. hampei* is necessary for setting up efficient control strategies in regions where populations have become resistant, as well as for populations not yet resistant. Among the population parameters relevant to pest control are the level of inbreeding and the level of gene flow. These can be inferred from degrees of population differentiation under models of population structure (Slatkin, 1993). High levels of inbreeding are expected to occur in *H. hampei*, since mating is supposed to be mainly between siblings, adult females do not seem to disperse far from the coffee fruits from which they emerge, adult males are flightless, and the sex-ratio is strongly female-biased, a feature typical of inbreeding insects (Hamilton, 1967). Direct estimations of inbreeding and gene flow require the availability of genetic markers and the analysis of their distribution within and among populations.

The present paper reports on the development of methods for allozyme electrophoresis in *H. hampei*, in order to obtain genetic markers for population studies. These were used to estimate genetic diversity, differentiation between populations, and inbreeding coefficients in this species.

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Table 1. Samples of *Hypothenemus hampei* analysed in the present survey.

Geographic origin	Status
Côte d'Ivoire	
Man (320 m)	random sample, natural population
Mont Toubou (1 200 m)	random sample, natural population
Mexico	
Tapachula, Chiapas	F5 laboratory strain reared in Noumea
New Caledonia	
Canala	random sample, natural population
La Foa	random sample, natural population
Poindimié	random sample, natural population
Touho	random sample, natural population
Ponérihouen 1	random sample, natural population
Ponérihouen 2	F11, resistant laboratory strain
Ponérihouen 3	F5 back-cross, resistant laboratory strain

Materials and methods

Sampling

Emerging females of *Hypothenemus hampei* were randomly taken from infested berries harvested at sites (coffee fields) in Côte d'Ivoire and New Caledonia (table 1). Two sites were sampled in Côte d'Ivoire, one a low altitude *Coffea canephora* plantation (ORSTOM station at Man; 320 m above sea-level), the second a mountain *C. arabica* plantation (ORSTOM collection at Mont Toubou; 1200 m). The sites in New Caledonia were *C. canephora* fields on the island's west coast (La Foa) where the habitat is considered to be protected, and on the east coast (Canala, Poindimié, Ponérihouen 1 and Touho), exposed to tropical winds and heavy rainfall. Samples Ponérihouen 2 and 3 and the sample from Mexico (table 1) consisted of females drawn from populations reared in the laboratory for several generations, in artificial conditions specified elsewhere (Brun *et al.*, 1993). All insects were sent alive by air from New Caledonia and

Table 2. Enzymes (with abbreviations and Enzyme Commission numbers (EC)) tested for activity on cellulose acetate gels in *Hypothenemus hampei*, and buffers yielding the best results. +, enzyme active; 0, no activity detected.

Enzyme		EC #	Activity	Buffer
AAT	aspartate aminotransferase	2.6.1.1	0	–
ACO	aconitase	4.2.1.3	0	–
ACP	acid phosphatase	3.1.3.2	0	–
ADA	adenosine deaminase	3.5.4.4	0	–
ADH	alcohol dehydrogenase	1.1.1.1	0	–
AK	adenylate kinase	2.7.4.3	0	–
AKP	alkaline phosphatase	3.1.3.1	+	TM
ALD	aldolase	4.1.2.13	0	–
ALDH	aldehyde dehydrogenase	1.2.1.5	0	–
AMY	α-amylase	3.2.1.1	0	–
AO	aldehyde oxidase	1.2.3.1	0	–
CAR	carbonate hydratase	4.2.1.1	0	–
CK	creatine kinase	2.7.3.2	0	–
DIA	diaphorase	1.6.*.*	0	–
EST	esterase (α-naphthyl-)	3.1.1.1	+	TM
EST-D	esterase (4-methylumbelliferyl-)	3.1.1.1	+	TM
FUM	fumarate hydratase	4.2.1.2	0	–
GDA	guanine deaminase	3.5.4.3	0	–
GDH	glucose dehydrogenase	1.1.1.47	0	–
α-GPD	α-glycerophosphate dehydrogenase	1.1.1.8	+	Phos
G-6-PD	glucose-6-phosphate dehydrogenase	1.1.1.49	0	–
GPI	glucose-phosphate isomerase	5.3.1.9	+	TM, CP
HBDH	β-hydroxybutyrate dehydrogenase	1.1.1.30	0	–
HK	hexokinase	2.7.1.1	0	–
IDH	isocitrate dehydrogenase	1.1.1.42	+	Phos, CP
LAP	leucine aminopeptidase	3.4.11.*	0	–
LDH	lactate dehydrogenase	1.1.1.27	0	–
MDH	malate dehydrogenase	1.1.1.37	+	Phos
ME	malic enzyme	1.1.1.40	+	Phos
MPI	mannose-phosphate isomerase	5.3.1.8	+	TM
NP	nucleoside phosphorylase	2.4.2.1	0	–
OPDH	octopine dehydrogenase	1.5.1.11	0	–
PEP	peptidases (1)	3.4.11.*	+	TM
6-PGD	6-phosphogluconate dehydrogenase	1.1.1.42	0	–
PGM	phosphoglucomutase	2.7.5.1	+	TM
SDH	sorbitol dehydrogenase	1.1.1.14	0	–
SOD	superoxide dismutase	1.15.1.1	+	TM
XDH	xanthine dehydrogenase	1.2.1.37	0	–
Prot	total proteins	–	0	–

(1) Five different substrates were tested (see text).

Côte d'Ivoire to France, where they were preserved in liquid nitrogen until electrophoretic analyses.

Processing

Each female *H. hampei* was ground in 5 µl buffer Tris-HCl-EDTA-NADP, pH 6.8 (Pasteur *et al.*, 1987) stained with bromophenol blue in an Eppendorff microtube, using a sealed micropipette tip as a pestle. About 1 µl of the liquid phase, used as the soluble enzyme extract, was loaded onto 5.7 cm×17 cm strips of cellulose acetate gel (Chemetron, Milano) using a draughtsman's pen. Three migration buffers were used: CP (10 mM NaH_2PO_4, 2.5 mM citric acid, pH 6.4), TM (50 mM Tris, 20 mM maleic acid, pH 7.8) and Phos (11.6 mM Na_2HPO_4, 8.4 mM NaH_2PO_4, pH 7.0) (Richardson *et al.*, 1986). Migration was induced by a constant potential, 200 V, corresponding to currents of 3 to 10 mA, depending on the buffer.

Forty-two enzymes (3-9 individuals per enzyme) were tested for their activity (table 2) using migration buffers CP and TM. The gels were stained for specific enzymes, following methods given in Pasteur *et al.* (1987) (AAT, ACP, AKP, AMY, EST, using radical α-naphthyl- as substrate, EST-D, using 4-methyl-umbelliferyl-), in Beaumont *et al.* (1980) (OPDH) and in Richardson *et al.* (1986) (all other enzymes). Staining solution (1-2 ml) was applied evenly onto each gel-strip, previously incubated in 0.5 ml Tris-HCl 0.1 M, pH 8.0 (for all stainings except phosphatases and esterases), Acetate-NaOH 0.15 M, pH 5.0 (ACP), Tris-NaCl-HCl 0.05 M, pH 8.4 (AKP), Phosphate Na/K 0.1 M, pH 6.5 (EST) or Acetate-Na 0.05 M, pH 6.0 (EST-D) (incubation buffers in Pasteur *et al.*, 1987). Total proteins were stained by incubating the gel for 1 hour in Coomassie Blue, then washing it for several hours in 5% acetic acid/5% ethanol solution until blue bands appeared against unstained background.

Five different oligopeptide substrates (Gly-Leu-Leu, Leu-Ala, Leu-Gly-Gly, Phe-Pro and Pro-Leu) were used to detect peptidase activity. Preliminary tests using these five substrates were made on TM gels only. Subsequent tests using only Leu-Ala as substrate were carried out on CP, Phos and TM gels.

Tests of polymorphism for enzymes found to be active were conducted using the migration buffers yielding the best results (activity, migration and resolution) (table 2), on total numbers of 38 to 223 insects from various origins: Côte d'Ivoire, plain and mountain, New Caledonia, east and west coasts, and Mexico.

Since the minimum quantity of enzyme extract necessary for each electrophoretic run was about one-fifth of the total volume available for each female, these could be characterized simultaneously for several, but not more than five enzyme stainings. Each individual was routinely typed for the following enzymes: EST-D and MPI, using TM migration buffer, GPI and IDH using CP buffer, and MDH using Phos buffer. GPI and IDH were stained simultaneously, by adding one drop of 1 mg/ml isocitrate solution to the reaction mixture for GPI.

Analysis of genotypic data

The genetic diversity was estimated over all samples and loci as

$$H = 1/l \Sigma_k (2N_k . (1-\Sigma_i x_i^2)/(2N_k-1))$$

where l is the number of loci, N_k the number of individuals characterized at locus k, and x_i the frequency of allele i at locus k.

Weir & Cockerham's (1984) parameters for *F*-statistics (Wright, 1969) were used for estimating the inbreeding coefficient (f) within populations and the degree of genetic differentiation between populations (θ). f corresponds to Wright's *Fis*, the correlation of alleles in an individual relative to alleles drawn at random from its own population. θ corresponds to Wright's *Fst*, the correlation of alleles within populations relative to alleles drawn at random from the total pool of populations. Two scales of observation were addressed: country (different samples in Côte d'Ivoire and New Caledonia), and world (all samples grouped by country).

The samples drawn from laboratory strains were not taken into account for the calculations of f. The laboratory sample from Mexico was included in the calculations of θ between countries, although some bias may be expected, because of the generally increased rate of genetic drift likely to occur in laboratory strains.

Results

Enzyme activity, resolution, and interpretation of zymograms

Nine enzymes (EST, EST-D, αGPD, GPI, IDH, MDH, ME, MPI and SOD) were active (table 2). Three other enzymes (AKP, PEP and PGM) showed a strong activity in some individuals, and no activity in other individuals run on the same gels. Highest PEP activity was obtained with Leu-Ala as substrate. The activity for all other enzymes and for general proteins was either very weak or not detected (table 2). All nine active enzymes and PEP (Leu-Ala) were tested on three buffers (CP, TM and Phos) for their resolution, that is, the sharpness of the bands of activity. The buffers eventually chosen as the result of a trade-off between optimal resolution and highest activity have been indicated in the last column of table 2.

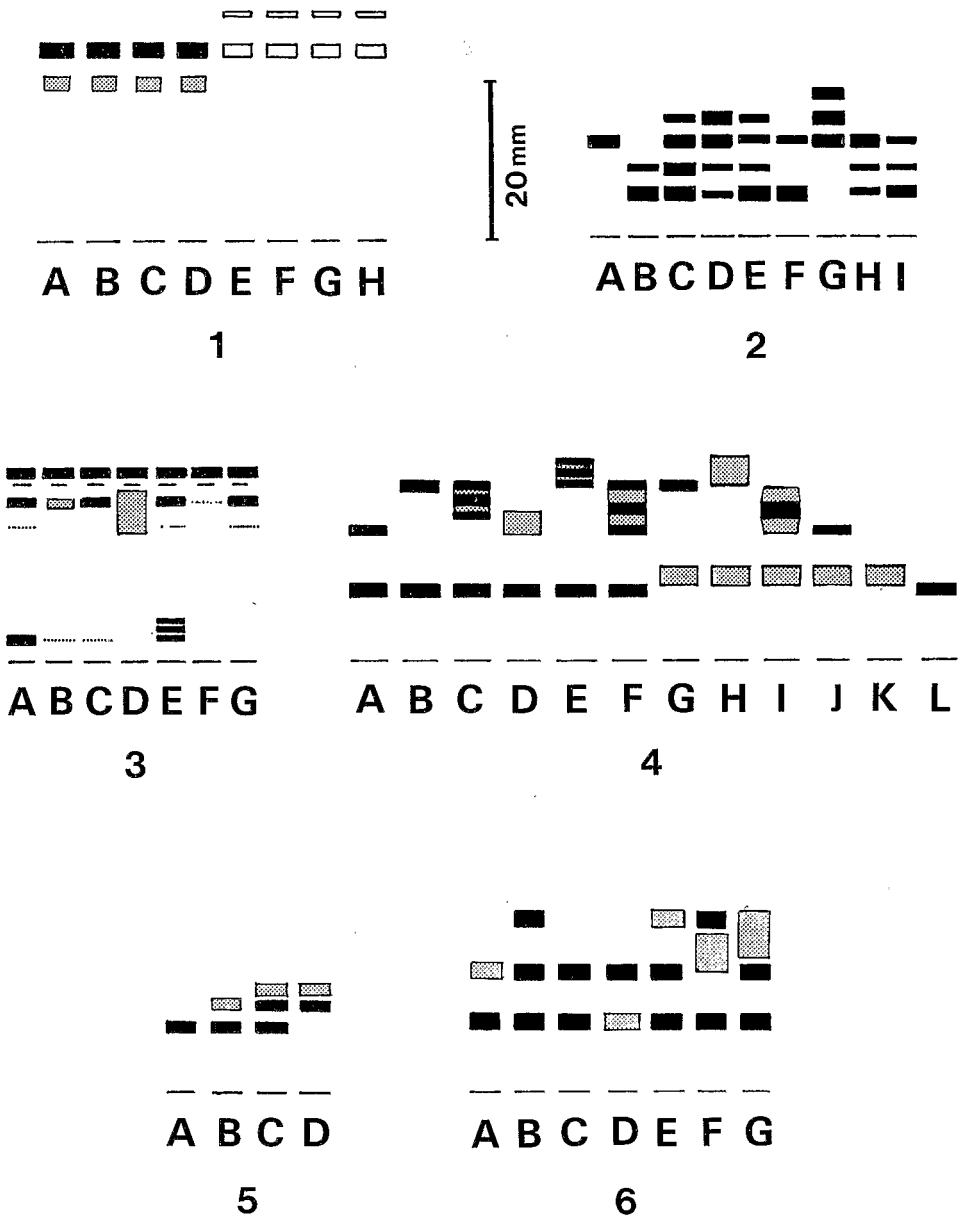
A detailed interpretation of the zymograms for each enzyme is presented below. Where explanations refer to figures, the latter included all phenotypes observed for the enzyme.

AKP

One single zone of AKP activity was present, but good resolution was not achieved with any buffer tested. In addition, variation in activity among individuals was considerable, with a large proportion of individuals showing no activity at all. AKP was not further considered in the study.

EST and EST-D

Two different esterase substrates were assayed: α-naphthyl acetate (αNA) and 4-methyl-umbelliferyl acetate (4MUA). Three zones of activity were scored (fig. 1), named EST-1, EST-2, and EST-3, in order of increasing mobility. EST-1 was αNA-specific, EST-3 was 4MUA-specific and EST-2 was active with either substrate. These differences in specificity for enzymes with different mobilities suggested that EST-1, EST-2 and EST-3 bands represent the products of three different esterase loci in *H. hampei*, namely, *Est*-1, *Est*-2 and *Est*-3.



Figs 1-6. Individual allozyme electrophoretic phenotypes observed in *Hypothenemus hampei* after specific staining for enzymes: 1, EST (lanes A-D: substrate αNA; E-H: substrate 4MUA); 2, αGPD; 3, GPI and IDH (IDH-1, IDH-2 and GPI zones of activity are in order of increasing mobility); 4, MDH (genotypic interpretations for MDH-2 phenotypes as following; A, J: *175/175*; B, G: *235/235*; C, *235/200*; D, *200/175*; E, H: *260/235*; F, I: *260/175*); 5, MPI (genotypic interpretations as following: A and B, *100/100*; C, *130/100*; D, *130/130*); 6, PEP(Leu-Ala). Shading indicates fainter activity. A to L: lanes loaded with enzyme extracts from each individual. Electrophoretic conditions: 1 hour at 200 V in buffers specified for each enzyme as in table 2.

αGPD

The αGPD patterns consisted of five bands with varying patterns of presence or absence in individuals tested (fig. 2). The observed polymorphism could be epigenetic:

1. Because αGPD is dimeric (Richardson *et al.*, 1986), the array of phenotypes presented in figure 2 cannot be interpreted under a simple Mendelian model.
2. When nicotinamide adenosine dinucleotide (NAD), a cofactor of αGPD (Harris & Hopkinson, 1976) was added to the grinding buffer, the faster bands tended to disappear while the slower bands became more active, suggesting that the enzyme migrates more slowly when it is saturated by NAD.

GPI

No polymorphism was observed for this enzyme (fig. 3, faster zone of activity), assumed to be encoded by one locus (*Gpi*).

Table 3. Enzyme phenotype frequencies in samples of *Hypothenemus hampei* from various locations. C, Canala; F, La Foa; Pd, Poindimié; P1-3, Ponérihouen; T, Touho; Ma, Man; Tb, Mont Toubou; Nd, origin not determined. Phenotype IN, inactive; UN, uninterpreted.

Phenotype		Number of individuals											
		New Caledonia								Côte d'Ivoire			Mexico
		C	F	Pd	P1	P2	T	P3	Nd	Ma	Tb	Nd	
Est-2	*100*	10	16	2	6	5	12	–	–	10	8	21	17
Est-3	*100*	10	10	2	3	2	12	–	–	–	5	18	17
Gpi	*100*	17	17	31	29	7	8	15	–	28	29	25	17
Idh-1	*100*	17	15	27	28	3	8	–	2	21	23	19	10
	R/100	0	0	0	0	0	0	–	0	0	1	0	0
	IN	0	0	0	1	0	0	–	0	0	0	2	0
Idh-2	*100*	17	17	28	24	3	10	21	2	28	33	27	10
	IN or UN	0	0	1	1	0	0	0	0	0	0	2	0
Mdh-1	*100*	4	9	14	27	–	8	3	–	8	5	8	13
	UN	4	7	2	3	–	0	0	–	2	3	1	2
Mdh-2	*260/235*	2	5	3	2	–	1	0	–	0	0	0	8
	235	5	7	12	17	–	7	0	–	7	3	2	2
	235/200	0	0	0	2	–	0	0	–	1	0	1	0
	235/175	0	0	1	4	–	0	1	–	0	1	0	1
	200/175	0	0	0	3	–	0	1	–	0	0	0	0
	175	0	0	0	2	–	0	1	–	1	2	6	0
	IN	0	0	0	1	–	1	0	–	0	0	1	0
Mpi	*100*	–	–	5	3	–	6	–	–	–	4	10	7
	130/100	–	–	0	0	–	1	–	–	–	1	0	0
	130	–	–	0	0	–	1	–	–	–	0	0	0

IDH

Two bands of activity were noted (IDH-1 and IDH-2) (fig. 3, below GPI bands). These were assumed to be determined by two loci, *Idh-1* and *Idh-2*. One heterozygote at locus *Idh-1* was detected (fig. 3, lane E; phenotype '*R/100*' in table 3). IDH-1 was inactive in some individuals which were nevertheless active for IDH-2 and GPI, stained on the same gel (fig. 3, lanes D, F, G), and IDH-2 patterns were unclear in some rare cases (fig. 3, lanes B, D, F; table 3).

MDH

Two different MDH enzymes were scored (MDH-1 and MDH-2) and were attributed to putative loci *Mdh-1* and *Mdh-2*, respectively. The more common MDH-1 phenotype (fig. 4, lanes A-F) was interpreted as that of individuals homozygous for an allele hereafter referred to as *100*. Another, less frequent phenotype was observed in most samples (fig. 4, lanes G-K; table 3). These individuals may be heterozygous for another allele encoding a faster isozyme. However, this interpretation must be regarded with caution, since MDH is dimeric in animals and plants (Richardson *et al.*, 1986) and the three-banded phenotypes characteristic of heterozygotes for a gene encoding a dimeric enzyme were not observed here, due perhaps to a lack of resolution. Furthermore, MDH is an enzyme known to be prone to artifacts due to modifying genes (Harry, 1983; Doebley *et al.*, 1986) or epigenetic phenomena (Richardson *et al.*, 1986). The phenotypes observed for MDH-2 (fig. 4) were those expected under the single-locus model for codominant genes encoding a dimeric enzyme and were assumed to be encoded by four alleles denominated as their electromorph's mobilities relative to that of electromorph MDH-1 *100*. The homozygous phenotypes for alleles *200* and *260*, and the heterozygous phenotypes *260/200* and *260/175* were not observed in the samples. MDH-2 was inactive in some individuals nevertheless active for MDH-1 (fig. 4, lanes K, L).

ME

Some ME activity was detected, yet MDH enzymes interfered with ME as supplementary stains, causing difficulties in interpretation. ME was not further considered in the study.

MPI

We assumed that the MPI phenotypes in *H. hampei* were determined by a single locus, *Mpi*, with two alleles present (fig. 5). Fainter bands were interpreted as artifactual.

PEP

Three zones of activity were present in PEP (Leu-Ala) zymograms (fig. 6), which could correspond to as many different loci. However, these presumptive loci were not expressed in all individuals (e.g. fig. 6, lanes A, C, D). Hence another interpretation would be that two loci encode PEP (Leu-Ala), with one locus monomorphic (PEP-1) and the second one, encoding faster allozymes (PEP-2), polymorphic. Under this two-locus model, some individuals would yet remain atypical (fig. 6, lanes F, G). Some individuals were inactive for PEP (Leu-Ala) while active for other enzymes.

Interpretations of PEP zymograms have proven difficult in other organisms (see Richardson *et al.*, 1986). Likewise, no clear interpretation could be drawn from the patterns observed in *H. hampei*.

PGM

Activity for this enzyme varied from one population to another, and also between individuals within the same population. In all individuals active, two zones of activity, close to one the other, were present. PGM being a monomeric enzyme (Richardson *et al.*, 1986), these patterns could be attributed either to the expression of two separate, monomorphic loci, or that of one single locus with two spots (main spot+secondary spot) for each individual's zymogram. Because of these difficulties in interpretation, PGM was not further considered in the study.

SOD

SOD activity was detected by the presence of clear bands against a coloured background. These bands almost did not migrate, whatever the buffer employed. Hence, no conclusion could be drawn about their monomorphism or polymorphism. SOD was not further considered in the study.

Genetic diversity

Among the 12 enzyme stainings found positive in *H. hampei*, 6 (EST, EST-D, GPI, IDH, MDH and MPI) exhibited at least partially interpretable zymograms. These were encoded by genes at presumptive loci (*Est*-1, -2, -3, *Gpi*, *Idh*-1, -2, *Mdh*-1, 2, and *Mpi*). Eight of these loci (all but *Est*-1) were tested for polymorphism. Only *Idh*-1, *Mdh*-2 *and Mpi* exhibited clear allozyme polymorphism. Loci *Est*-2 (107 individuals tested), *Est*-3 (62), and *Gpi* (223) showed sample monomorphism. It was not clear whether *Idh*-2 and *Mdh*-1 polymorphisms are genetically determined or artefactual.

In table 3 are reported all results as phenotype (presumed genotype) frequencies in samples of individuals characterized routinely for enzymes EST-D, GPI, IDH, MDH and MPI. Over the 7 presumed loci (*Est*-2, *Est*-3, *Gpi*, *Idh*-1, *Idh*-2, *Mdh*-2 and *Mpi*) encoding enzymes whose zymograms could lead to orthodox interpretations (which was basically the case for *Idh*-2, in spite of some rare individuals whose genotype could not be established), only two (*Mdh*-2 and *Mpi*) could be considered polymorphic, i.e. that the largest allele frequency was less than 0.95.

In table 4 are reported the values of allele frequency and genetic diversity at loci *Mdh*-2 and *Mpi*, estimated from the total sample in order to yield values tentatively representative of the species over its current distribution range. We obtained the following values of genetic diversity *sensu lato*:

1. Percentage of loci polymorphic, for which the frequency of the most common allele was less or equal to 0.95: $P=0.28$.

Table 4. Allelic frequencies at loci *Mdh*-2 and *Mpi* in *Hypothenemus hampei* (estimated from total sample). N, sample size. x_i, allelic frequency; monolocus genetic diversity: $h=2N.(1-\Sigma_i x_i^2)/(2N-1)$.

Locus	N	Allele	x_i	h
Mdh-2	109	*260*	0.10	0.457
		235	0.72	
		200	0.04	
		175	0.14	
Mpi	38	*130*	0.05	0.100
		100	0.95	

Table 5. *Hypothenemus hampei*. Values for parameters f and θ (Weir & Cockerham, 1984) estimated at the local scale (locations in New Caledonia and Côte d'Ivoire) and at the world scale (New Caledonia, Côte d'Ivoire, Mexico). Multilocus values calculated after Weir & Cockerham (1984) ± standard deviations, estimated using the jackknife on the sets of monolocus values per sample. N, average number of individuals per locus per sample.

Scale		Locus		
		Mdh-2	*Mpi*	multilocus
New Caledonia	N	14.6	5.3	
	f	0.087	0.653	0.298 ± 0.283
	θ	0.039	−0.010	0.023 ± 0.024
Côte d'Ivoire	N	7.5	–	
	f	0.679	–	–
	θ	0.070	–	–
World	N	36.3	12.7	
	f	0.221	0.489	0.273 ± 0.134
	θ	0.137	−0.020	0.110 ± 0.079

2. Mean number of alleles per locus, whose frequency was more or equal to 0.05: $A=1.43$.
3. Average genetic diversity: $H=0.080$.

Population structure

A high degree of inbreeding was observed within populations (fields) from New Caledonia and Côte d'Ivoire (table 5). Discrepancies were noted in θ estimates, with negative values at locus *Mpi* (an artefact of the method for estimating θ when the value of the latter is close to zero; Weir & Cockerham, 1984) and large, positive values at locus *Mdh*-2.

Discussion

A variety of factors other than genetic polymorphism can result in enzyme electrophoretic variation: these include environmental effects, protein degradation, uncontrolled variation in gel quality, and other artefacts in staining (Oxford, 1975; Harris & Hopkinson, 1976; Richardson *et al.*, 1986). The banding variation observed in *H. hampei* for enzymes AKP, αGPD, IDH-2 and PEP possibly fell into some of these categories. Other enzymes exhibited individual banding patterns that could be interpreted genetically. However, some doubt remains, particularly for MDH-1 and possibly for MPI, and crossing experiments are required to definitively establish the Mendelian determination of the phenotypes observed. Electrophoretic variation for one enzyme, MDH-2, fulfilled the following criteria:

1. Consistency of the banding patterns with the known quaternary structure of the enzyme.
2. Presence of the phenotypes expected to be in non-marginal proportions according to a Mendelian model.

Electrophoretic variation of IDH-1 also fitted at least the first of these criteria. Yet both IDH-1 and MDH-2 were inactive in some individuals found active for other enzymes. This could be caused by uneven grinding leaving unground the specific tissues or organites in which these enzymes are active, or by variations in activity related to the physiological status of the individuals.

Electrophoretic variation of IDH, MDH and MPI has a simple genetic basis in other insects (e.g. Loukas *et al.*,

1979), supporting the inference that electrophoretic variation for these enzymes in *H. hampei* is due to genetic polymorphism at Mendelian loci. Hence, the presumptive loci *Mdh*-2 and *Mpi* were used as genetic markers in coffee berry borer populations. *Idh*-1 was not polymorphic enough to serve as a suitable genetic marker for population studies.

The mating system in *H. hampei* was expected to consist mainly of full-sib crosses, since females have been reported to mate within the coffee bean in which they developed, to leave the bean after mating, and each to oviposit in a new coffee bean (Bergamin, 1943). The possibility of some outbreeding cannot yet be ruled out, since multiple infested coffee beans have been observed in some circumstances (Sladden, 1934). The high values for the fixation index (f) reported in the present study indicated that inbreeding is a major process in *H. hampei*, although a certain degree of outbreeding was demonstrated, for 100% full-sib mating would have lead to $f=1$ (no heterozygotes in the population at equilibrium if mutation is neglected), which was not the case here.

One of us (D.P. Gingerich, unpublished data) independently estimated the inbreeding coefficient at the local scale (coffee plantation) on the basis of the distribution of the presumed *RR* and *RS*, resistant, and *SS*, susceptible genotypes of female individuals, determined according to their survival to discriminating doses of endosulfan (a cyclodiene). Values estimated for f were of 0.408 ± 0.050, which was roughly in accordance with the allozyme data-based estimates reported here.

Under Wright's island model of population structure, *Fst* is tied to *Nm*, the effective number of migrants per generation at equilibrium, by the relation $Fst = 1/(4Nm+1)$ (Wright, 1969). This result has been shown to be robust in actual situations (Slatkin, 1993). The θ values given in the last column of table 5 were used to estimate *Nm* according to the above equation. $Nm = 10.6$ individuals per generation between localities in New Caledonia and $Nm = 2.0$ individuals per generation between countries. Such gene flow may be mediated by inadvertent transport at the worldwide scale, which has been documented (see Bergamin, 1943). Because the calculations included one laboratory strain, it is likely that some bias due to genetic drift led to a somewhat overestimated degree of differentiation between countries. On the other hand, these *Nm* values correspond to theoretical equilibrium, which is a heavy assumption considering that the arrival of *H. hampei* in the countries sampled is recent (Giordanengo, 1992).

The value of genetic diversity in *H. hampei* was significantly lower than the average in Coleoptera ($H \pm \mathrm{SE} = 0.191 \pm 0.032$) (Graur, 1985) and also below the average in insects, *Drosophila* spp. (Diptera: Drosophilidae) excluded ($P = 0.351 \pm 0.187$; $H = 0.089 \pm 0.060$) (Nevo *et al.*, 1984). Bottleneck effects due to the recent introduction of *H. hampei* in Mexico, Côte d'Ivoire and New Caledonia and the high level of inbreeding evidenced in this species may suffice to account for relatively low values of genetic diversity. However, the first of these hypotheses would hold true only if native populations of *H. hampei* were proven to be genetically more diverse. It would thus be of interest to compare these findings with estimates of the genetic diversity of populations from north-eastern Africa, the apparent geographic origin of this species.

The number of polymorphic enzyme loci available in *H. hampei* is insufficient for a reliable, accurate picture of its population structure, because of the level of inter-locus variation in the estimates of *Fst*, as usually encountered in allozyme data-based population studies (e.g. Krafsur *et al.*, 1992). Nevertheless, allozyme genetic markers may prove useful for investigating the determination of sex, still unclear in *H. hampei*, for paternity testing, and as alternative, neutral markers of population structure relative to the presumably highly selected GABA-receptor gene involved in resistance to cyclodiene insecticides (ffrench-Constant *et al.*, 1994).

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