Biodiversity and Biogeography of an Important Inbred Pest of Coffee, Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae)

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ABSTRACT Amplified fragment length polymorphism (AFLP) fingerprinting was used to examine the genetic variability and biogeography of the most important insect pest of coffee, *Coffea arabica* L., the coffee berry borer, *Hypothenemus hampei* (Ferrari). *H. hampei* samples ($n = 101$) from 17 countries on three continents were examined. Only 26 unique fingerprints (haplotypes) were discovered among all samples. Genetic variability was extremely low (10% average polymorphism per sample), but genetic differentiation was high ($\Phi_{ST} = 0.464$). The distribution of the fingerprints and their genetic relatedness to each other suggested that a West African source population invaded both Asia and America. Three distinct lines entered the Americas through either separate introductions or a single introduction of multiple lines. At least two were first introduced to Brazil and subsequently dispersed throughout the Americas. The third was discovered only in Peru and Colombia. Observations were consistent with the high rate of inbreeding suspected of this pest. With such high inbreeding, undesirable mutations, such as those conferring insecticide resistance, might rapidly become homozygous in *H. hampei*. However, the low genetic variability observed also suggests that this pest may lack the genetic variability necessary to respond to an intensive control strategy.

RESUMEN La técnica AFLP fue usada para generar huella dactilares genéticas examinando la variación genética y la biogeografia de la plaga mas importante del cultivo del café, *Coffea arabica* L., la broca del café *Hypothenemus hampei* (Ferrari). Muestras de *H. hampei* ($n = 101$) provenientes de 17 países en tres continentes fueron examinadas. Solo 26 huellas dactilares (haplotipos) fueron descubiertas. La variación genética fue extremadamente baja (10% promedio de polimorfismos por muestra), pero la diferenciación genética fue alta ($\Phi_{ST} = 0.464$). La distribución de las huellas dactilares y la relación genética entre ellas sugirieron que una población originaria del Oeste de Africa que debió haber invadido tanto Asia como América. Tres lineas genéticas distintas entraron a las Américas, ya sea a través de la introducción independiente de varias lineas o a la introducción única de lineas multiples. Al menos dos lineas fueron inicialmente introducidas al Brasil y posteriormente se dispersaron por todas las Américas. La tercera linea fue decubierto solo en Perú y Colombia. Estas observaciones fueron consistentes con la alta tasa de endogamia con la que se sospecha que este insecto posee. Si esto es cierto, algunas mutaciones no deseadas, asi como aquellas que confieren resistencia a insecticidas, podrían volverse homozigóticas rápidamente. Sin embargo, la baja variación genética observada también podría estar sugiriendo que esta plaga carece de la variación genética necesaria para responder a una estrategia de control intensiva.

KEY WORDS amplified fragment length polymorphism, DNA fingerprinting, pseudo-arrhenotoky, invasive species, Broca

COFFEE, *Coffea arabica* L., is a major source of foreign income for nations in the tropics. Unfortunately, a small scolytid, the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), has invaded nearly all coffee-producing nations and has severely limited the quantity and quality of coffee production (Le Pelley 1968, Bustillo et al. 1998). Adult female *H. hampei* bore into developing coffee berries where they deposit 50–75 eggs. The developing larvae feed on the coffee beans inside the berry (Bustillo et al. 1998). Like other seed-feeding scolytids, *H. hampei* has a female biased sex ratio; each female produces nine daughters for every son (Bergamin 1943, Kirkendall 1993, Bustillo et al. 1998, Nor-

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mark et al. 1999). Males are smaller and flightless, and unmated females are sterile (Barrera et al. 1995). Therefore, it is generally accepted that females mate with their male siblings before dispersing to infest other coffee berries (Bustillo et al. 1998). Inbreeding in *H. hampei* is further accentuated by the pseudo-arrhenotokous cytogenetics of this insect in which both sexes are diploid, but males transmit only their maternally derived chromosomes to their offspring (Brun et al. 1995).

Such inbreeding predicts that *H. hampei* reproduces through matrilineal lines of descent. If true, such matrilineal lines have been introduced into most coffee-producing countries only within the past 60 yr (Bergamin 1946, Bustillo et al. 1998). Furthermore, if only a few matrilineal lines have been introduced, it is possible that the genetic diversity among populations within these regions is extremely low. Therefore, it might be possible to determine the biogeography of this pest if sufficient between-line genetic variation exists. Results of previous investigations that used allozymes (Borsa and Gingerich 1995) and insecticide resistance (Gingerich et al. 1996) as genetic markers have indicated that this possibility is likely. In addition, a recent analysis of *H. hampei* mitochondrial DNA sequence suggested that only two inbred lines, both originating from East Africa, have colonized the world (Andreev et al. 1998). However, the number of polymorphic loci examined in these studies was limited to two allozymes, the *Resistance to dieldrin* locus, the intergenic spacer region (ITS2), and a mitochondrial locus (CO1).

In the present investigation, the amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) was used to generate DNA fingerprints of 101 *H. hampei* samples collected from 17 countries in Asia, Africa, and America. This permitted an examination of hundreds of loci for the presence of dominant DNA polymorphisms among a relatively wide distribution of samples. Our objectives were to identify DNA polymorphisms among *H. hampei* and to use these to determine population substructure and provide inferences with regard to the biogeography of this pest. Extremely low genetic variability was detected, especially within locations. However, the variation was sufficient to identify 26 unique matrilineal lines among the samples. An analysis of these haplotypes suggested that at least three invasions of this pest occurred in the Americas and at least two of these occurred in Brazil.

Materials and Methods

Insect Samples. *H. hampei* samples (n = 101) were obtained from 17 countries on four continents (Table 1). Two types of samples were used. Multiple bean samples consisted of *H. hampei* captured from a pool of infested beans collected from single locations. *H. hampei* adults emerging from each of these samples were bulked and maintained at −80°C. Single bean samples consisted of all stages of *H. hampei* within single coffee beans. Infested coffee beans were collected in the field and placed separately in containers. Each bean was then dissected, and all the insects within the bean were stored in absolute ethanol. Total genomic DNA was extracted from each sample using the DNeasy tissue kit (QIAGEN, Valencia, CA). DNA quality was examined by agarose gel electrophoresis, and its concentration was determined using a Hoefer Scientific TKO 100 Mini-fluorometer. Genomic DNA samples were maintained at −70°C.

DNA Fingerprinting. AFLP was performed using the AFLP Analysis System II for small genomes (Invitrogen, Carlsbad, CA) and [^{33}P]dATP end-labeled primers. Forty selective amplifications were performed on two *H. hampei* DNA samples from Colombia by using 40 combinations of *Eco*RI and *Mse*I primers: *Eco*RI primers (E) with two additional nucleotides at their 3′ end (E-AA, E-AC, E-AG, E-AT, and E-TG) and *Mse*I primers (M) with three additional nucleotides at their 3′ ends (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, and M-CTT). One sample was of bulked field-collected *H. hampei*. The other was a single bean sample (CO03) that was used as a standard in all subsequent AFLP experiments. Six selective primers (E-AT combined with M-CAT, M-CTA, M-CTC, and M-CTG, and E-TG combined with M-CAT and M-CTG) were chosen on the basis of these results for the analysis of 73 samples collected from 12 countries (Table 2). Two selective primer combinations (E-AT/M-CTC and E-AT/M-CTG) were used with all 101 samples. Amplified fragments were separated by gel electrophoresis for 2 h at 50 V in 6% Long Ranger (Cambrex Bio Science Rockland, Inc., Rockland, ME) denaturing polyacrylamide gels. After electrophoresis, the gels were dried and exposed to Biomax MR X-ray film (Eastman Kodak, Rochester, NY) for 48–72 h for autoradiography. Banding patterns were visually converted into digital fingerprints. To avoid artifacts, bands that were not recognized by two independent observers were eliminated from the analysis. The presence or absence of the bands in each sample was then recorded as a 1 for presence and as a 0 for absence. All comigrating bands were assumed to be identical in sequence.

Statistical Analysis. Digital fingerprints were used to calculate percentage of polymorphism among countries and primer combinations, as well as to infer population genetic structure and to perform a neighbor-joining cluster analysis. The percentage of polymorphism among primer combinations was obtained as the proportion of the number of polymorphic bands to the total number of fragments generated by AFLP with each primer combination in all countries. Percentage of polymorphism within countries was determined as the proportion of the number of polymorphic bands to the total number of fragments generated by AFLP in all samples within each country.

Population genetic structure was resolved using an analysis of molecular variance (AMOVA) as implemented in Arlequin version 2.0 (Excoffier et al. 1992). This analysis considered only those fingerprints that were derived from single bean samples collected at locations that had at least two samples (Brazil, Colombia, Costa Rica, Ecuador, Honduras, Mexico, Peru,

Table 1. Worldwide collection of *H. hampei* samples used in this investigation

Sample	Country/region	Latitude/longitude	Yr	Type[a]
BR01-08	Brazil/Piracicaba	9.0 S/72.8 W	2000	Sb
BR0-14	Brazil/La Cumbre	7.9 S/42.0 W	2000	Sb
CO0-05	Colombia/Risaralda	4.9 N/75.6 W	2000	Sb
CO06	Colombia/Caldas	5.0 N/75.6 W	2000	Sb
CO07, 08	Colombia/Antioquia	5.2 N/75.1 W	2000	Sb
CO0-10	Colombia/Nariño	1.6 N/77.1 W	2000	Sb
DR01	Dom. Rep./Santiago	19.6 N/70.9 W	2000	Sb
EC01, 05	Ecuador/ Manabi	1.3 S/80.6 W	2000	Sb
EC0-04	Ecuador/Pichincha	1.0 S/79.8 W	2000	Sb
HO01, 03, 07	Honduras/Cortes	13.8 N/87.2 W	2000	Sb
HO02, 0-05	Honduras/Ilama	15.1 N/88.2 W	2000	Sb
HO06	Honduras/Pta. Blanca	16.4N/86.4W	2000	Sb
ME0-15	Mexico/Chiapas	16.5N/91.8W	2000	Sb
NI01	Nicaragua	Unknown	1999	Mb
NI02	Nicaragua/El Nispero	11.7 N/86.1 W	1999	Mb
NI03	Nicaragua/El Tuma	13.1 N/85.8 W	1999	Mb
NI04	Nicaragua/Matagalpa	12.9 N/86.0 W	1999	Mb
PE01, 02	Peru/Paucartambo	13 S/71 W	2000	Sb
PE03	Peru/Lamas	6.4 S/76.5 W	2000	Sb
PE04, 05, 07	Peru/Cajamarca	7.2 S/78.5 W	2000	Sb
PE06	Peru/Chanchamayo	13.7 S/75.8 W	2001	Sb
PE08, 09	Peru/Amazonas	6.3 S/77.4 W	2001	Sb
PE10	Peru/El Dorado	4.9 S/79.0 W	2001	Sb
SA01	ElSalvador/Cuscatlan	14.0 N/90.0 W	2000	Sb
JA01	Jamaica/S. Catherine	17 N/76 W	2000	Mb
JA02	Jamaica/S. Andrew	18 N/76 W	2000	Mb
CA01	Cameroon/Nkolbison	3.9 N/11.6 E	1999	Mb
CA02	Cameroon/Atok	4.0 N/12.8 E	1999	Mb
ET01	Ethiopia/Jima	9.5 N/38.1 E	2001	Sb
ET02	Ethiopia/Tepi	7.4 N/35.4 E	2001	Sb
KE01, 02	Kenya/Thika	1.6 S/37.0 E	2000	Mb
UG01, 03	Uganda/Mukono	0.3 N/32.5 E	1997	Mb
UG02	Uganda/Mubende	0.6 N/31.4 E	1997	Mb
UG04	Uganda/Bushenyi	0.5 S/30.2 E	1997	Mb
UG05	Uganda/Budadiri	1.2 N/34.3 E	1997	Mb
UG06	Uganda/Luwero	0.9 N/32.5 E	1997	Mb
UG07	Uganda/Nyenje	0.4 N/32.7 E	1997	Mb
UG08	Uganda/Sembabule	0.1 S/31.5 E	1997	Mb
UG09	Uganda/Iganga	0.7 N/33.2 E	1997	Mb
UG10	Uganda/Nakikoma	0.8 N/31.3 E	1997	Mb
UG11	Uganda/Mbale	0.7 S/30.6 E	1997	Mb
UG12	Uganda/Masaka	0.3 S/31.7 E	1997	Mb
IA01, 02	Indonesia/Malang	8.0 S/113.5 E	2000	Sb
IA03, 04	Indonesia/Jember	8.2 S/113.7 E	2000	Sb
IN01	India/Kodagu	12.3 N/75.9 E	2000	Mb
IN02	India/Siddapur	12.3 N/75.9 E	2000	Mb
IN03	India/Kutta	12.3 N/75.9 E	2000	Mb
IN04	India/Suntikoppa	12.5 N/75.8 E	2000	Mb
IN05	India/Perumbalai	12.0 N/98.0 E	2000	Mb
IN06	India/Bangalore	13.0 N/77.6 E	2000	Mb
CR0-04	Costa Rica/Heredia	10.0 N/84.1 W	2001	Sb

[a] Mb, females from multiple beans; Sb, females from single beans.

Ethiopia, and Indonesia). Locations were treated as populations. Under the null hypothesis of no population substructure, all locations were first tested as one group. To establish significance for the molecular fixation index Φ, individual fingerprints were permuted among locations and the fixation indices recomputed. The data were permuted and recomputed 1,023 times. The *P* value associated with the fixation index was the probability of having a more extreme variance component and Φ-statistic than the observed values by chance alone. Tests of alternative hypotheses were constructed by distributing the locations among the number of hypothesized subpopulations and then proceeding as before. For Φ_{CT}, locations were permuted across subpopulations, and for Φ_{SC} individual finger-

Table 2. DNA polymorphisms detected by AFLP per country

Country	*n*	Total no. bands	No. polymorphic bands	% polymorphism
BR	14	201	21	10.4
CA	1	197		
CO	1	192		
EC	5	194	1	0.5
HO	7	194	3	1.6
IA	4	195	1	0.5
IN	6	192	1	0.5
JA	2	191	0	0
ME	15	192	2	1.0
NI	5	198	7	3.5
UG	12	210	15	7.1
SA	1	195		

Country abbreviations are the same as in Table 1.

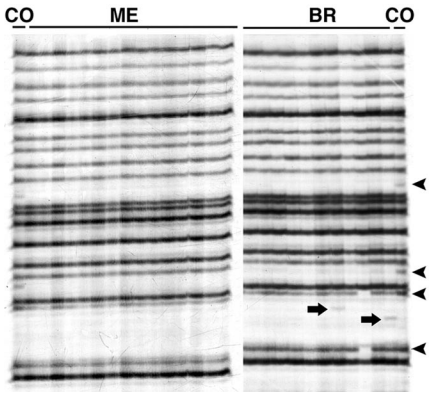

Fig. 1. Two gels showing *H. hampei* AFLP-based DNA fingerprints for the Colombian standard CO03 (CO), and samples from Brazil (BR) and Mexico (ME). AFLP–PCR was performed using a single selective primer combination (E-TG/M-CTG). Four DNA polymorphisms are visible (arrowheads). A few AFLP artifacts (arrows) also are visible on these gels. These were absent in all other samples and were not scored as polymorphisms.

prints were permuted across locations but within the same subpopulation.

Neighbor-joining cluster analysis was performed using the software Phylogenetic Analysis Using Parsimony (PAUP 4.0b10 Altivec) (Swofford 1998). Bootstraping was performed with 2,000 resamplings and 10,000 replications with the total number of pairwise character differences distance setting. The Ethiopian samples were used as the out-group.

Table 3. DNA polymorphisms detected by AFLP per primer combination

Primer combination	Total no. bands	No. polymorphic bands	% Polymorphism
E-AT/M-CAT	45	4	8.9
E-AA/M-CTA	41	8	19.5
E-AT/M-CTC	40	15	37.5
E-AT/M-CTG	40	14	35.0
E-TG/M-CAT	43	9	20.9
E-TG/M-CTG	23	2	8.7
Total	232	52	22.4

Results

To determine which AFLP-selective primers revealed DNA polymorphisms among *H. hampei* samples, 40 selective primer combinations were tested with two *H. hampei* DNA samples from Colombia. Thirty-seven of these combinations produced DNA fingerprints of both samples (data not shown), revealing an average of 31.2 ± 8.5 bands per combination, 1,149 monomorphic bands, and seven polymorphic bands. Six of the primer combinations that detected polymorphisms between the Colombian samples were then used with 73 samples collected from 12 countries (Table 2; Fig. 1). Additional polymorphic bands were discovered, and Uganda had 2 to 7 times more variability than any region, except Brazil (Table 2). Al-

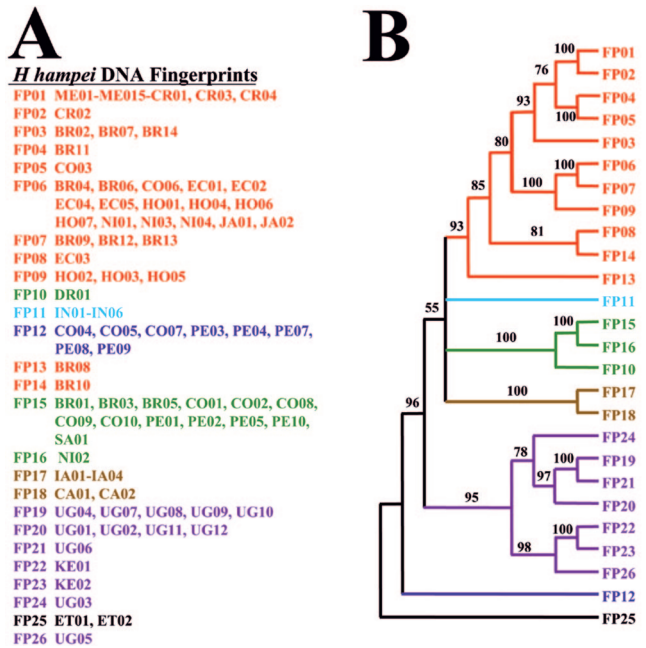

Fig. 2. *H. hampei* AFLP fingerprints (A) and neighbor-joining analysis (B). The distribution of fingerprints (FP01–FP26) among the samples listed in Table 1. Fingerprints (haplotypes) and samples are color coded according to groups (B). Six major groups were identified. The Ethiopian samples (black) were used as the out-group. The East African group is purple, the Indonesian-Cameroon group is brown, and the Indian group is light blue. The three Latin American groups are colored red (clade 1), dark blue (clade 12), and green (clade 15).

Table 4. Hierarchical analysis of variance for nine locations with no grouping (1) or with two groups (Ethiopia vs. all others, 2)

Group	Variance	df	Variance	% total	P^a	Φ-Statistics
1	Among locations σ_a^2	8	0.216	46.46	0.000	
	Within locations σ_c^2	59	0.249	53.54	0.000	$\Phi_{ST} = 0.464$
	Total	67	0.465			
2	Among groups σ_a^2	1	0.102	18.17	0.224	$\Phi_{CT} = 0.181$
	Among locations within groups σ_b^2	7	0.209	37.37	0.000	$\Phi_{SC} = 0.456$
	Within locations σ_c^2	59	0.249	44.46	0.000	$\Phi_{ST} = 0.555$
	Total	67	0.560			

[a] Probability of obtaining a random value higher than the observed value.

though mean percentage of polymorphism within countries was only 2.9 ± 3.6, the percentage of polymorphism between samples across all primer combinations was nearly 10-fold higher (Table 3). Two selective primer combinations revealed 56% of the polymorphic bands. These primers were used to examine all 101 samples from all locations. They revealed only 26 unique DNA fingerprints (Fig. 2A), consisting of 51 monomorphic fragments and 29 polymorphic fragments. Together, these observations indicated that AFLP fingerprinting had revealed DNA polymorphisms that might be useful in examining the biogeography of this pest.

Analysis of Molecular Variance. To test for population substructure in *H. hampei*, AMOVA was performed using the AFLP fingerprinting data. Considering that this insect shows high inbreeding and sibmating, new mutations are expected to become fixed or eliminated in only a few generations. Therefore, all bands in each AFLP fingerprint were assumed to be homozygous in each sample so that each fingerprint could be analyzed as a haplotype. To best meet the assumptions, only the single bean samples were included in this analysis because the multiple bean samples were more likely to mask polymorphisms within the populations. Rather than partitioning the variance of allele frequencies and correlating genes as for *F*-statistics with codominant markers, in this Φ-statistics analysis the genotypic variance was partitioned and the genotypes were correlated. *H. hampei* seemed to have great genetic differentiation between Ethiopia and the rest of the world, and very great genetic differentiation within populations and among locations (Table 4). Under the null hypothesis of no substructure where all of the samples are tested as if they comprised one population, 46.5% of the variance was due to differences in fingerprints among, rather than within locations ($\Phi_{ST} = 0.46$). The null hypothesis of no substructure was not supported (10^3 permutations, $P < 0.0001$).

These observations were clearly consistent with the expectation that *H. hampei* is a highly inbred species. However, a high value for Φ_{ST} may result when one population is very different from the others and all populations show relatively little within-population variation. Furthermore, the two Ethiopian samples had 10 private alleles (Fig. 3), whereas no other country had more than three private alleles (10 countries had no private alleles). Therefore, we tested the alternative hypothesis of two subpopulations (one Ethiopian, and the other, all remaining locations), and found that 18.2% of the variance was due to the difference between the two groups, 37.4% to differences among locations within groups, and 44.5% to the differences within locations ($\Phi_{ST} = 0.56$). The separation of the Ethiopian samples into a subpopulation, despite the number of private alleles, was only weakly supported by the permutation test (10^3 permutations, $P > 0.24$). We concluded that the disproportionate number of Ethiopian private alleles was due to the proximity of those samples to the pest's center of origin.

Distribution of Inbred Lines. Psuedo-arrhenotoky in *H. hampei* (Brun et al. 1995) combined with the inbreeding that was evident from the AMOVA indicated that the assumption of each unique fingerprint representing a different inbred line was appropriate in the previous analysis (Fig. 2A). The greatest number

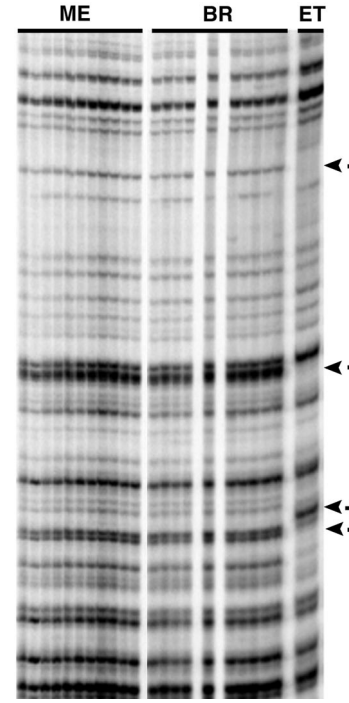

Fig. 3. Polymorphisms in the Ethiopian AFLP fingerprints. One private allele (+) was present only in the Ethiopian fingerprint and three others (−) were absent from the Ethiopian fingerprint when compared with Mexican (ME) and Brazilian (BR) fingerprints with primer combination E-AT/M-CTG.

of lines (eight) was found in East Africa, nearest the presumed center of origin, consisting of samples from Ethiopia ($n = 2$), Kenya ($n = 2$), and Uganda ($n = 12$). Multiple lines were discovered in Brazil (seven, $n = 14$), Central America and the Caribbean (seven, $n = 19$), Colombia (four, $n = 10$), Ecuador (two, $n = 4$), and Peru (two, $n = 10$). A single fingerprint was discovered in Indonesia ($n = 4$), another single fingerprint was discovered in India ($n = 6$), and another single fingerprint was discovered in Cameroon ($n = 2$). Only four lines (FP01, FP06, FP12, and FP15) were discovered in more than one country. These were found only in Latin America and composed 76% of all Latin American samples.

Neighbor-Joining Analysis. To analyze the data for genetic relatedness among lines, neighbor-joining cluster analysis was performed on all 26 matrilineal lines (Fig. 2B). We assumed that AFLP fingerprints revealed sufficient variability to determine the genetic relatedness among the lines and that each colonizing population was derived from a single source population. The Asian and American colonization most likely occurred as separate introductions because these continents are widely separated. Therefore, we reasoned that the separation between the Asian samples and the American samples would be one measure of how separate introductions might appear at a single colonizing location such as Brazil. We also reasoned that the relatedness among Kenyan and Ugandan samples might indicate how much genetic diversity could be expected in a source population. To root the analysis, the Ethiopian haplotype (FP25) was used as the outgroup because it was both collected nearest the presumed center of origin and because of the disproportionate number of private alleles associated with this haplotype.

Neighbor-joining separated the 26 fingerprints into six major groups (Fig. 2B). The Kenyan and Ugandan haplotypes (FP19–24 and 26) clustered into one group that was clearly separated from all other samples. Four other groups were placed together. The Indonesian haplotype and the Cameroon haplotype formed one of these groups, and the Indian haplotype formed another. Latin American fingerprints formed three groups. Two of these were grouped with the Indian, and Indonesian-Cameroon samples. Of these two groups, the largest contained FP06, FP01, and 10 other haplotypes (Fig. 2, red group), the other contained FP15 and two other haplotypes (Fig. 2, green group). The remaining American group consisted of a single haplotype, FP12 (Fig. 2, dark blue group), which was equally distant from the other American haplotypes and the East African haplotypes.

Discussion

The number of polymorphisms observed among *H. hampei* was extremely low. This was consistent with the expectation that *H. hampei* is an inbreeding pest. This observation also was consistent with the observations of Andreev et al. (1998), who found extremely low genetic variability. The low number of polymorphisms attests to the reproducibility and reliability of the polymerase chain reaction (PCR)–AFLP technique to produce DNA fingerprints of *H. hampei* because much greater rates of polymorphism would be expected if the technique were generating spurious banding patterns. Furthermore, in spite of the limitations associated with the dominant nature of AFLP polymorphisms, the genetic variability revealed by this analysis was greater than that observed in any previous investigation of *H. hampei* (Borsa and Gingerich 1995, Andreev et al. 1998, Gauthier and Rasplus 2004). In fact, even though seven polymorphic microsatellites were recently revealed on *H. hampei*, we found this number very low for a population that was expected to contain the highest genetic variability, Kenya and Ethiopia (Gauthier and Rasplus 2004). Nonetheless, the present investigation may have underestimated the true genetic variability of this insect because the data analysis assumed that cosegregating AFLP fragments derived from separate samples were identical in sequence and that all polymorphic bands are homozygous when present.

Species that are spread over large areas usually fragment into subpopulations when mating between individuals within a given location are more likely than mating between individuals from different locations (Hartl and Clark 1997, Hanski 1999). Using codominant markers, the genetic differentiation between subpopulations can be measured by the fixation index F_{ST} (Wright 1921). A species such as *H. hampei* that seems to reproduce through highly inbred lines would be expected to show great genetic differentiation both among locations and within populations due to the establishment of multiple but highly differentiated pure lines at each location. Wright (1951) considered an F_{ST} value between 0.15 and 0.25 as an indication of great genetic differentiation and a F_{ST} value >0.25 as evidence of very great genetic differentiation. Fixation indices >0.3, although typical in studies of uniparentally inherited organelle DNA, are rare in studies of nuclear DNA unless the organism is highly inbred or has recently experienced a severe bottleneck. By these criteria, *H. hampei* seemed to have great genetic differentiation between Ethiopia and the rest of the world, and very great genetic differentiation within populations and among locations (Table 4). However, it is important to consider that in the present investigation rather than partitioning the variance and correlating genes as for *F*-statistics with codominant markers, Φ-statistics analysis was used to partition the genotypic variance of allele frequencies and correlate the genotypes. Therefore, even though the proportion of variance due to different levels was informative and its significance was meaningful, the estimated Φ_{ST} inferred in this investigation cannot be directly compared with the F_{ST} calculated by other investigations (Borsa and Gingerich 1995, Gingerich et al. 1996).

An inbreeding strategy combined with exclusion of the paternal chromosomes might be expected to result in multiple inbred lines because neutral mutations are likely to become fixed, creating a novel matrilineal line, within two generations. Once fixed, the new

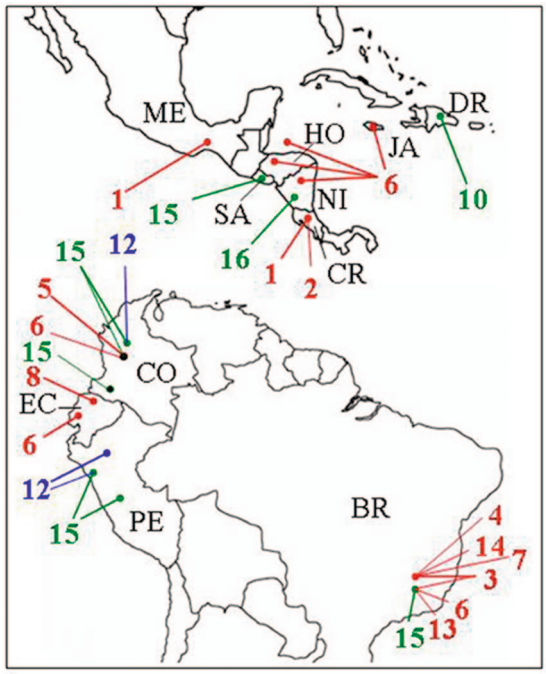

Fig. 4. Distribution of AFLP fingerprints in Latin America. The color of each fingerprint is consistent with the color-coding shown in Fig. 2. The country abbreviations are the same as in Table 1.

allele will persist until a reversion mutation restores the original sequence. Because the probability of restoration is less than the probability of any other type of mutation, differences accumulate in the form of new matrilineal lines over time. Therefore, as a practical consideration, mutations conferring insecticide resistance in *H. hampei* would be expected to rapidly become homozygous and spread as a single line (Brun et al. 1995). This poses a real challenge in the management of this pest because control in affected areas often relies on chemicals. Resistance to endosulfan, the insecticide most commonly used to control *H. hampei*, has been reported in New Caledonia (Brun et al. 1989) and Colombia (Gongora et al. 2001). Another important consideration, however, is that the lack of genetic variability in *H. hampei* might increase its risk of extinction (Frankham 1995, Saccheri et al. 1998, Hanski 1999), or in other words, the genetic plasticity that might allow another species to overcome an extremely intensive management strategy is evidently very low in *H. hampei.*

The neighbor-joining cluster analysis found evidence for three separate introductions of *H. hampei* into the Americas (Figs. 2 and 4). Each putative introduction would have involved a related group of beetles represented by the three major American groups (Fig. 2): the red group, containing FP01, FP06 and nine others; the green group, containing FP15, and two others; and the dark blue group, consisting of FP12. The red and green groups were more closely related to the orange group from Cameroon than the purple group from Kenya and Uganda. That supports the idea that the beetle was first introduced to Brazil from West Africa (Bergamin 1946). The dark blue group (FP12), however, is clearly distantly related to the red and green groups. This haplotype represents the most compelling evidence for multiple introductions into America, both because of its distance from other American lines and the absence of a related line among the Brazilian samples. Previous reports have suggested that Peruvian *H. hampei* were initially introduced from Brazil (Amaral 1963). Therefore, we suspect that our failure to detect the FP12 haplotype in Brazil was due to the collection of few samples from few locations in an area that contains a relative abundance of genetic variability. Nonetheless, this analysis does not eliminate the possibility that there may have been an introduction of *H. hampei* directly into Peru or Colombia.

The genetic analysis of *H. hampei* biogeography largely supports certain contentions present in historical accounts of this pest's spread throughout coffee-producing regions. First, greater genetic variability was discovered in Africa. This supports the contention that this insect's center of origin is Africa. Second, greater genetic diversity was observed in Brazil than other Latin American countries. This observation supports the suggestion that *H. hampei* was first introduced to the Americas by shipments of infested seeds to Brazil (Bergamin 1946), making Brazil the American center of origin. Third, dispersal from Brazil to other parts of Latin America was supported by the presence of lines FP06 (present among 16 samples) and FP15 (present among 13 samples) in Brazil and other Latin American nations (Fig. 4). Furthermore, the clear genetic relatedness of other lines in Latin America (red and green groups, Figs. 2 and 4) indicates that as the beetles invaded other Latin American coffee-growing regions from Brazil, the founders in each region were a subsample of the genetic diversity present in Brazil. Therefore, with regard to the red group in Figs. 2 and 4, FP01 was detected only in Costa Rica and Mexico, FP02 was detected only in Costa Rica, FP05 was detected only in Colombia, FP08 was detected only in Ecuador, and FP09 was detected only in Honduras. A similar pattern was observed among the lines that compose the green group in Figs. 2 and 4. Although clearly related to haplotypes present in Brazil and other parts of Latin America, FP10 was only discovered in the Dominican Republic, and FP16 was discovered only in Nicaragua. Fourth, the relationships among the fingerprints and their distribution support the supposition that separate introductions were responsible for *H. hampei* in Indonesia and India (Corbett 1933, Sreedharan et al. 1994). Fifth, the separation of East African haplotypes from all other haplotypes is consistent with the suggestion that introductions of this pest into both Asia and America occurred by way of West Africa (Bergamin 1946). In fact, the close similarity between fingerprints discovered in Indonesia and Cameroon suggested that the beetles that invaded Indonesia had a West African origin.

We noted a few inconsistencies between the genetic analysis and previous reports. Andreev and collaborators suggested that Jamaican *H. hampei* were more closely related to African *H. hampei* than other American *H. hampei* (Andreev et al. 1998). Our study, however, clearly showed the Jamaican line (FP06) present in other American nations. This inconsistency suggests that additional sampling may discover a distinct AFLP haplotype associated with the more African mitochondrial type in Jamaica. Neighbor-joining cluster analysis grouped Colombian samples in independent groups with samples from Peru, Brazil, and Ecuador. This suggests that *H. hampei* probably invaded Colombia from multiple introductions rather than a single Ecuadorian invasion as postulated previously (Bustillo et al. 1998). Further research will address the origin of these multiple introductions of *H. hampei* into Colombia as well as the possible patterns of its dispersion throughout Latin American countries and the Caribbean.

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