GENETIC FINGERPRINTING OF THE SCREWWORM (DIPTERA:CALLIPHORIDAE) INFESTATION IN NORTH AFRICA BY MITOCHONDRIAL DNA MARKERS

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ABSTRACT

A screwworm sample, *Cochliomyia hominivorax* (Coquerel), collected from Libya in 1990, during the final phase of the eradication program, was analyzed for mitochondrial DNA (mtDNA) RFLP patterns. The goal was to determine whether these fertile flies represented reintroduction from either a new source or contaminants in the shipments of sterile flies from the Mexican screwworm factory, (used for eradication of flies in Libya) or progeny of the original introduction in 1988. Samples of laboratory-strains originating from South America, Mexican factory, Costa Rica, and a 1989 collection from Libya were also analyzed. These patterns were compared with the previously published patterns of 30 laboratory strains originating from Mexico, Central America and Jamaica and earlier sample of flies from Libya. The restriction patterns (with *Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I and *Sst*I) of 1990-Libyan screwworm were similar (mitochondrial similarity, F=0.97) to those of South American flies but different from those of Mexico (F=0.53), Jamaica and Central America (F=0.71). The *Hpa*I and *Ssp*I digests of DNA amplified by primer flanking mitochondrial CO-I to CO-II region produced patterns which were similar in Libyan and South American flies but different from those of Mexican and Costa Rican samples. These data confirmed that the flies collected in Libya in 1990 did not originate from Mexico, Central America or Jamaica. Our data suggest some region(s) of South America could be the likely source of screwworm infestation in North Africa. However, it would be necessary to analyze several geographical samples from South America to confirm this tentative conclusion.

Key Words: Screwworm, *Cochliomyia hominivorax*, PCR, mitochondrial DNA RFLP, geographical variation

RESUMEN

Fueron analizados los patrones RFLP de DNA mitocondrial (mtDNA) de una muestra de gusanos barrenadores del ganado, *Cochliomyia hominivorax* (Coquerel), colectada en Libia en 1990 durante la fase final del programa de erradicación. El objetivo fue determinar si las moscas habían sido reintroducidas de una nueva fuente o

si eran contaminantes en los envíos de moscas estériles de la fábrica de gusanos barrenadores del ganado de México (usados para la erradicación de las moscas en Libia) o si eran parte de la progenie de la introducción original de 1988. También fueron analizadas muestras de cepas de laboratorio originales de América del Sur, de la fábrica de México, de Costa Rica, y de una colección de Libia. Estos patrones fueron comparados con los patrones previamente publicados de 30 cepas de laboratorio de México, America Central y Jamaica y con muestrtas anteriores de moscas de Libia. Los patrones de restricción (con *Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I y *Sst*I) de gusanos de tornillo de Libia de 1990 fueron similares (similitud mitocondrial, F=0.97) a aquellos de las moscas de América del Sur, pero diferentes de los de México (F=0.53), Jamaica y América Central (F=0.71). Los análisis de restricción de DNA con *Hpa*I y *Ssp*I amplificados por "primer flanking" a la región mitocondrial CO-I a CO-II originaron patrones similares a los de las moscas de Libia y América del Sur y diferentes de los de las muestras de México y Costa Rica. Estos datos confirman que las moscas colectadas en Libia en 1990 no se originaron en México, América Central o Jamaica. Nuestros datos sugieren que alguna región o regiones de América del Sur podrían ser la fuente de la infestación de Africa del Norte. Sin embargo, sería necesario analizar varaias muestras geográficas de América del Sur para confirmar esta conclusión tentativa.

The New World screwworm (NWS), *Cochliomyia hominivorax* (Coquerel) (Diptera:Calliphoridae), is a major agent of myiasis of livestock and wildlife in the New World (Knipling & Rainwater 1937). Originally, it was widely distributed from the southern U.S. to northern Chile. However, it was eradicated in the United States by August, 1982, and in Mexico in February, 1991, (FAO 1992) by the sterile insect technique (SIT), as described by Knipling (1955). In March, 1988, NWS were detected in North Africa (Libya) causing wound infestations in livestock and humans. This caused great concern that the screwworm might spread to Europe, throughout Tunisia and Egypt, and up the Nile into Sub-Saharan Africa. In 1991, the United Nations Food and Agriculture Organization (FAO) declared eradication of screwworm from Libya by the sterile insect technique.

The source of the 1988 screwworm infestation in Libya is not known. Taylor et al. (1991) compared the mitochondrial DNA (mtDNA) restriction pattern of the Libyan flies with the previously published patterns (Roehrdanz, 1989) of 30 strains originating from Mexico, Central America and Jamaica. They concluded that the 1989 collection of Libyan flies had a unique mitochondrial genotype and that the 1988 infestation in Libya did not originate from Jamaica, Mexico or Central America.

During May, 1991, we received a sample of flies from Libya (collected in November, 1990, near the end of the eradication program). We also received flies from South America (Brazil) and Costa Rica. We analyzed mitochondrial DNA of these flies to determine whether or not: 1) the latest sample from Libya represented a new introduction, 2) PCR-based DNA markers could be used to distinguish Libyan flies from South American, Central American, Mexican and Jamaican flies, and 3) there were any genetic differences between Costa Rican samples collected in 1986 and 1991.

Materials and Methods

Collection of Samples

The 1990 Libyan sample was from a strain which originated from three egg masses collected from wounds on sheep near Tripoli, Libya, in November of 1990, and

that were maintained in Libya for five generations. A sample of pupae was transported from Libya to Fargo and the adults were used for mtDNA and PCR studies. Frozen samples of the 1989 Libyan flies (LIB-89) were also analyzed. These flies originated from a laboratory strain established from two eggs masses collected near Tripoli in October, 1989. Flies were frozen for mtDNA analysis each generation up to 15 generations. The South American sample (BRA-90) was collected in August, 1990, by exposing a wounded cow to feral flies in Rio de Janeiro, Brazil. About 500 larvae were collected from the wound, reared to pupae and transported to Fargo. The adults were combined and frozen samples from the ensuing F_5 and F_{10} generations were used for DNA analysis. The Costa Rican sample of 1991 (COR-91) was from a composite laboratory strain. It originated from eggs laid by twenty-two individual flies collected on liver. Of these isofemale lines, only sixteen were established successfully in the laboratory. These were later combined to establish a composite strain. After 2-3 generations of culture in the laboratory, adults were frozen for mtDNA analysis. Flies originating from the 1986 collection from Costa Rica (COR-86) were analyzed earlier by Roehrdanz (1986). We compared mtDNA data of COR-91 with that of COR-86. The OW-87 strain was used as a reference standard for comparison of our results with those published by Roehrdanz (1989). This strain is currently being mass-produced in Mexico for use in the sterile insect release program in Central America. The OW-87 was also released in Libya. This strain originated from egg masses collected in the vicinity of Orange Walk, Belize, in October, 1986 (refer to Taylor et al. 1991).

Restriction Analysis of mtDNA.

Total cellular DNA from individual flies was isolated by the method of Boyce et al. (1989) with minor modifications. Individual flies were homogenized in 700 µl of CTAB buffer (0.1 M tris HCl, pH 8.0, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB - hexadecyl trimethylammonium bromide, 0.2% 2-mercaptoethanol), incubated at 65°C for 45 minutes and centrifuged. The supernatant was extracted with an equal volume of chloroform:isoamyl alcohol and DNA was precipitated by adding 2/3 vol of isopropanol. The DNA pellet was washed with cold 70% ethanol, vacuum dried and dissolved in 50 µl 1X TE (10 mM tris, 1 mM EDTA, pH 8.0) containing 10 µg/ml DNAse free RNAse A.

DNA samples were digested separately with 15 restriction endonucleases and electrophoresed in 1% agarose gels in TBE buffer. Two molecular size markers, 1 Kb ladder and lambda *Hind*III (purchased from BRL) were used in each gel for estimation of size of restriction fragments. The DNA gels were blotted on to nylon membranes according to Reed & Mann (1985). Purified mtDNA from laboratory reared screwworm pupae of OW-87 (courtesy of Dr. Roehrdanz) was used to probe Southern blots. The hybridization mixture contained ^{32}P labeled probe and molecular size markers in 0.5 M phosphate buffer (pH 7.0), 1 mM EDTA, 1% BSA and 7% SDS. After overnight hybridization at 65°C, membranes were washed and exposed to X-ray film. The mitochondrial similarities (*F*) among geographical samples, based on the proportion of identical fragments, were calculated by the method of Nei & Li (1979).

PCR Amplification.

Total DNA prepared from individual adults served as a source of mtDNA template for the PCR. We used sequences from conserved regions spanning 12SrRNA, 16SrRNA, isoleucine t-RNA, CO-I and CO-II subunit genes of *Drosophila* (Simon et al. 1991) and the honeybee (Hall & Smith 1991) as primers (Table 1). These oligonu-

TABLE 1. LIST OF PRIMERS USED TO AMPLIFY CERTAIN REGIONS OF MITOCHONDRIAL GENOME OF SCREWWORM, *COCHLIOMYIA HOMINIVORAX*, BY POLYMERASE CHAIN REACTION.

Primer Set No.		Sequence	Ref. Fly Sequence
1	►	5' AAA CTA GGA TTA GAT ACC CTA TTA T 3'	12sai (Simon et al. 1991)
	◄	5' ATG TTT TTG ATA AAC AGG CG 3'	16sa (Simon et al. 1991)
2	►	5' CGC CTG TTT ATC AAA AAC AT 3'	16sar (Simon et al. 1991)
	◄	5' CTC CGG TTT GAA CTC AGA TC 3'	16sbr (Simon et al. 1991)[1]
3	►	5' ATT TAC CCT ATC AAG GTA A 3'	t-Iso (Simon et al. 1991)
	◄	5'CGG GCG ATG TGT ACA TAA TT 3'	12sfi (Simon et al. 1991)
4	►	5' ATT TAC CCT ATC AAG GTA A 3'	t-Iso (Simon et al. 1991)
	◄	5' AGG GTA TCT AAT CCT AGT TT 3'	12sair (Simon et al. 1991)
5	►	5' TTG ATT TTT TGG TCA TCC AGA AGT 3'	CO-I (Hall & Smith, 1991)
	◄	5'GAT CAA TAT CAT TGA CC 3'	CO-II (Hall & Smith, 1991)

[1]Modified 16sbr

cleotide primers were synthesized (National Biosciences) and used for amplifications of DNA segments. The PCR was performed as described by Simon et al. (1991). Each reaction volume was 100 µl and contained about 0.1 µg of total cellular DNA, 10 µl PCR buffer (10X), 150 nmole $MgCl_2$, 20 pmole of each primer, 20 nmole each of dATP, dCTP, dGTP and dTTP, and 2.5 units Taq polymerase. The reaction profile consisted of 2 cycles of 95°C for 2 min, 50°C for 2 min, 72°C for 4 min; 32 cycles of 93°C for 1 min, 50°C for 1 min, 72°C for 4 min, and a final cycle of 93°C for 1 min, 50°C for 1 min and 72°C for 10 min.

Aliquots (10 µl) of PCR product were digested with eight enzymes (*Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I, *Sst*I, *Ssp*I). The digests along with a molecular size marker (∅X174 *Hae*III digest) were electrophoresed in 3.0% agarose and stained with ethidium bromide. The sizes of fragments were estimated against the molecular size marker.

RESULTS

Mitochondrial DNA Restriction Patterns

Data in Table 2 show the mitochondrial DNA restriction fragments observed with 15 enzymes. Table 3 and Figs. 1 to 5 summarize the restriction fragment patterns in LIB-89, LIB-90, BRA-90 and COR-91 strains. The designations for restriction patterns correspond to those of Roehrdanz (1989). When our estimates of the sizes of one or more fragments in a specific pattern differed slightly (500 bp or less) from those reported by Roehrdanz (1989), we maintained the same letter designation but indicated it by parenthesis. Three new patterns, not reported in earlier studies (Roehrdanz 1989, Taylor et al. 1991) are indicated with asterisks (Tables 2 and 3).

The three new patterns included: pattern F for *Msp*I in LIB-89, LIB-90 and BRA-90 (Figs. 1 & 2); G for *Hind*III in COR-91 (Fig. 3); and C for *Pvu*II in BRA-90 (not shown). These three patterns were not reported before in LIB-89 (Taylor et al. 1991)

TABLE 2. ESTIMATES OF FRAGMENT SIZES OF MITOCHONDRIAL DNA RESTRICTION PATTERN OBSERVED FOR EACH ENZYME IN GEOGRAPHICAL SAMPLES OF SCREWWORM. FOR OTHER PATTERNS NOT SHOWN HERE, REFER TO ROEHRDANZ (1989).

Enzyme	Haplotype[1]	Restriction Fragment Sizes (Kb)
*Eco*RI	A	9.0, 3.5, 1.5, 1.2, 1.05
*Hind*III	A	6.8, 4.2, 3.3, 0.52, 0.5, 0.45
	D	6.8, 4.0, 3.8, 0.52, 0.5, 0.45
	G[1]	5.5, 3.2, 2.8, 1.3, 0.52, 0.5, 0.45
*Hae*III	A	7.5, 4.8, 2.0, 1.1, 0.8
	B	7.5, 7.0, 1.1, 0.8
*Msp*I	A	5.0, 4.9, 4.2, 1.4
	B	6.1, 5.0, 4.2
	F[1]	4.9, 4.8, 4.2, 1.4, 0.3, 0.2
*Eco*RV	A	6.6, 5.0, 2.9, 1.5
	B	6.6, 5.0, 4.5
*Pvu*II	A	7.8, 3.0, 2.9, 0.87, 0.57
	C[1]	8.8, 3.0, 2.9, 0.87, 0.57
*Hpa*I	A	9.5, 6.5
	B	16
*Sst*I	A[2]	9.0, 6.0, 0.7
	B[2]	6.4, 5.2, 3.8, 0.7
*Xho*I	A	16
*Xba*I	A	16
*Pst*I	A	16
*Fnu*dII(BstuI)	A	13, 2.7
*Fnu*4HI	A	2.7, 2.6, 2.1, 1.9, 1.8, 1.4, 1.3, 0.8, 0.73, 0.4
	C	2.7, 2.6, 2.1, 1.9, 1.4, 1.3, 1.2, 0.8, 0.73, 0.6, 0.4
*Sau*96I	A	5.8, 4.7, 2.0, 1.6, 1.1, 0.4, 0.3
*Scr*FI	A	5.8, 4.8, 2.6, 1.4, 0.85, 0.75
	C	8.2, 4.8, 1.4, 0.85, 0.75

[1]Indicates that these haplotypes have not been reported in previous publications (Roehrdanz, 1989, Taylor et al., 1991).
[2]The haplotypes underlined indicate that fragment sizes are different from those reported in the previous publications.

or in thirty screwworm lines (Roehrdanz 1989). Patterns A and F of *Msp*I were not distinguishable (Fig. 1) until the gels were run for a relatively longer period of time (Fig. 2). In gels run for normal time (when the 0.6 Kb marker band of the molecular size marker, lambda *Hind*III, approaches close to the anodic end of the gel), the two *Msp*I fragments, 4.9 and 4.8 Kb of the pattern A appeared as a single band as did the 5.0 and 4.9 Kb fragments of pattern F (Fig. 1). These bands are better resolved in long-run gels as shown in Fig. 2. The other patterns shown in Figs. 4 and 5 include patterns A and B of *Sst*I, A of *Sau*96I, *Pvu*II and *Eco*RI, A and B of *Hpa*I, and A and B of *Eco*RV.

TABLE 3. COMPARISON OF MTDNA RESTRICTION GENOTYPES IN GEOGRAPHICAL SAMPLES OF SCREWWORM.

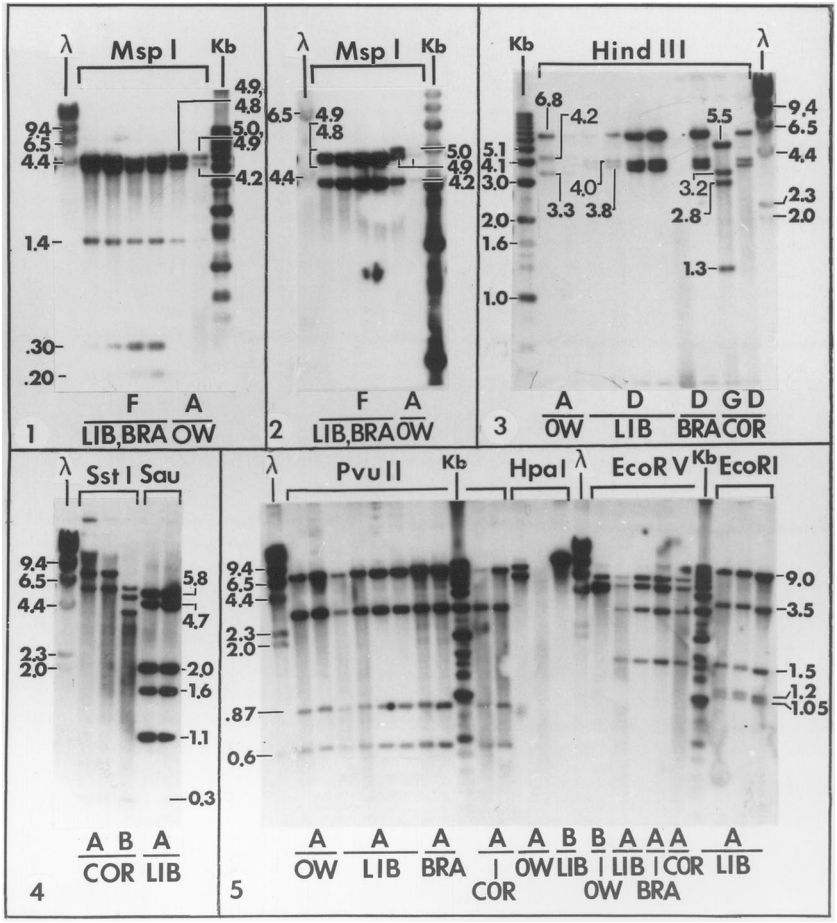
	Haplotypes in Screwworm Populations[1,2]					
Enzyme	LIB-91	LIB-89	BRA-91	OW-87	COR-91	COR-86
*Eco*RI	A	A	A	A	NT	A
*Hind*III	D	D	D	A	D,G*	A
*Hae*III	B	B	B	B	A	A
*Msp*I	F*	F*	F*	A	A	B
*Eco*RV	A	A	A	B	A	A
*Pvu*II	A	A	A,C*	A	A	A
*Hpa*I	B	B	B	A	NT	A
*Sst*I	(B)	(B)	(B)	(A)	(A),(B)	A
*Xho*I	A	A	A	A	A	A
*Xba*I	A	A	A	A	A	A
*Pst*I	A	A	A	A	A	A
*Fnu*dII(BstuI)	A	A	A	A	A	A
*Fnu*4HI	C	C	C	A	A	A
*Sau*96I	A	A	A	A	A	A
*Scrf*I	C	C	C	A	C	C

[1]The asterik (*) indicates that these haplotypes have not been reported in previous publications (Roehrdanz, 1989; Taylor et al., 1991).
[2]The haplotypes in parenthesis indicate that fragment sizes are different (refer to Table 2) from those reported in the previous publications. The data on CR-86 is from Roehrdanz (1989).

The mtDNA restriction patterns in Libyan flies for 7 (47%) enzymes (*Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I and *Sst*I) were similar to those of South American (BRA-90) flies (mitochondrial similarity, F=0.97). These patterns were different from those of flies from Mexico, Jamaica and Central America. The South American sample was polymorphic for *Pvu*II sites (patterns A and C), whereas, the Libyan samples (LIB-89 and LIB-90) were fixed for pattern A. Similarly, COR-91 was polymorphic for *Hind*III (pattern D and G) and *Sst*I (patterns A and B). The COR-91 flies differed from Libyan (F=0.71) and South American flies (F=0.69) with *Fnu*4HI, *Hae*III and *Msp*I. There were more differences in restriction endonuclease recognition sites between the laboratory-reared Mexican strain and the Libyan (F=0.53) and South American (F=0.52) flies than between the latter two and flies from Costa Rica (COR-91). The patterns obtained for *Bstu*I, *Eco*RI, *Pst*I, *Pvu*II, *Sau*96I, *Xba*I and *Xho*I were not diagnostic for any population.

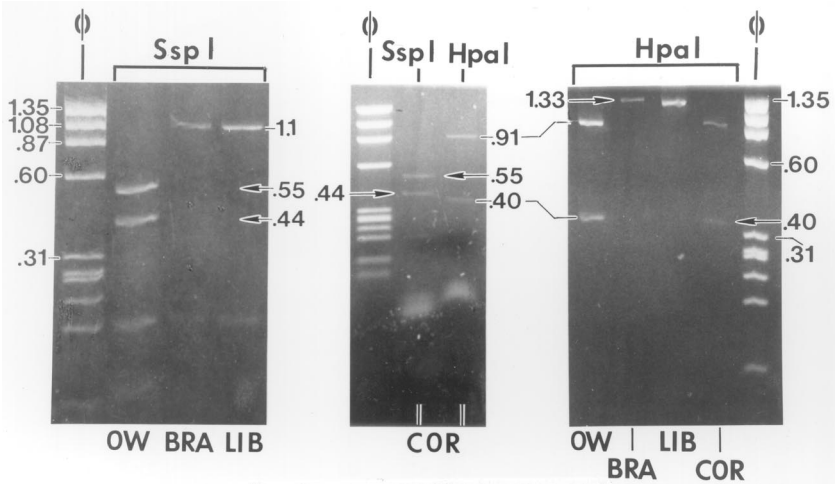
PCR Amplified DNA Restriction Patterns

The results in Table 4 show the fragments obtained by digestion of amplified DNA samples. Of the 8 restriction enzymes tested on the DNA samples amplified by primer #5 (Table 1), flanking region between CO-1 and CO-II, only two (*Hpa*I and *Ssp*I produced diagnostic patterns (Figures 6 to 8). The patterns obtained with *Hpa*I and *Ssp*I were similar in Libyan and South American flies (Fig. 6). These patterns were differ-



Figures 1-5: **1 & 2**) The mtDNA restriction fragment size differences between the *Msp*I A pattern of Mexican (OW) and the pattern F of Libyan (LIB) and South American (BRA) flies. Fragments, 5.0 and 4.9 bands of the pattern A and 4.9 and 4.8 of the pattern F can only be distinguished in Fig. 2 (from long-run gel) but not in Fig. 1 (gel run for normal period of time). Kb ladder (KB) and lambda *Hind*III digest were used as molecular size markers; **3**) *Hind*III restriction patterns showing the type A in Mexican (OW) the type D in Libyan (LIB) and South American (BRA) and types G and D in Costa Rica (COR); **4**) The patterns A and B of *Sst*I in Costa Rica (COR) and the pattern A of *Sau*96I in Libyan (LIB) flies; **5**) Restriction patterns for *Pvu*II, *Hpa*I, *Eco*RI and *Eco*RV.

ent from those obtained in Costa Rican and Mexican samples. *Hpa*I produced 2 fragments (0.91 and 0.38 Kb) in Costa Rican and Mexican strains but one uncut fragment (1.37 Kb) each in Libyan and South American strains. *Ssp*I produced 4 fragments (0.55, 0.44, 0.15, 0.08 Kb) each in Costa Rican and Mexican strains but 3 fragments (1.08, 0.15, 0.08 Kb) each in Libyan and South American strains. The patterns obtained with six other enzymes were similar in all the 4 samples tested. The digestion



Figures 6-8. Restriction patterns obtained by digestion of DNA samples amplified by PCR primer pair 5 (see Table 1). *Hpa*I and *Ssp*I patterns of LIB and BRA are different from those of OW and COR.

of DNA amplified by primer #1 flanking region between 12SrRNA and 16SrRNA region and primer #2 flanking internal sequences within 16SrRNA gene (Table 1) produced similar patterns for 8 restriction enzymes in flies from the four populations. The bands of DNA amplified by primers #3 and #4 were either weak or not reproducible.

Discussion

Our first goal was to determine whether the LIB-90 sample (collected toward the end of the screwworm eradication program in Libya) represented a new infestation. Our data on mtDNA RFLP and the PCR-amplification patterns showed no evidence for new infestation(s). The mitochondrial genome of LIB-90 was almost identical (F=0.97) to that of LIB-89. However, occasional reinfestation from the original geographical source cannot be ruled out.

Roehrdanz (1989) identified 16 mitochondrial genotypes in 30 laboratory lines originating from Mexico, Jamaica and Central America. Although restriction studies on LIB-89 by Taylor et al. (1991), did not reveal any new patterns, the composite haplotype of LIB-89 differed from those 16 reported in Jamaican, Mexican and Central American flies by Roehrdanz (1989). They designated this new haplotype as 17 and determined the cladistic relationships by the unrooted dendrogram. Although our results on restriction patterns (of LIB-89 and LIB-90 strains) for *Msp*I and *Sst*I differed (due to the improvement in resolution of fragments by our electrophoretic and southern blotting methods) from those of Taylor et al. (1991), they do not change the overall relationship between haplotype 17 and the other 16 haplotypes. The mitochondrial genome of South American (BRA-90) and LIB-90 flies is almost identical (F=0.97), therefore, BRA-90 flies can tentatively be designated as haplotype 17.

We also wanted to determine whether some region of South America could be the source of the original 1988 infestation in Libya. To address this type of question, analysis of mtDNA RFLP is considered to be useful for monitoring population dispersal and maternal lineages, because the mtDNA is maternally inherited without recombi-

TABLE 4. RESTRICTION FRAGMENT PATTERN OF PCR AMPLIFIED DNA OF SCREWWORM SAMPLES.

Enzyme	Restriction Fragment Sizes Primers (refer to Table 1) 1	2	5
*Eco*RV			
LIB-90	1.35	.59	.85, 0.5
BRA-90	1.35	.59	.85, 0.5
OW-87	1.35	.59	.85, 0.5
COR-91	—	—	—
*Fnu*4HI			
LIB-90	1.36	.46	1.36
BRA-90	1.36	.46	1.36
OW-87	1.36	.46	1.36
COR-91	—	—	—
*Hind*III			
LIB-90	.59, .48, .22	.59	1.36
BRA-90	.59, .48, .22	.59	1.36
OW-87	.59, .48, .22	.59	1.36
COR-91	—	—	—
*Hpa*I			
LIB-90	1.35	.58	1.37
BRA-90	1.35	.58	1.37
OW-87	1.35	.58	.91, .38
COR-91	—	—	.91, .38
*Msp*I			
LIB-90	1.22	.59	1.16
BRA-90	1.22	.59	1.16
OW-87	1.22	.59	1.16
COR-91	—	—	—
*Scrf*I			
LIB-90	1.35	.59	1.22
BRA-90	1.35	.59	1.22
OW-87	1.35	.59	1.22
COR-91	—	—	—
*Ssp*I			
LIB-90	.71, .26, .07	.29, .21	1.08, .15, .08
BRA-90	.71, .26, .07	.29, .21	1.08, .15, .08
OW-87	.71, .26, .07	.29, .21	.55, .44, .15, .08
COR-91	—	—	.55, .44, .15, .08
*Sst*I			
LIB-90	1.35	.59	1.17
BRA-90	1.35	.59	1.17
OW-87	1.35	.59	1.17
COR-91	—	—	—

nation (Avise et al. 1987). Paternal contributions to the mtDNA gene pool are rare or non-existent (Lansman et al. 1983). Although several geographical samples from South America (we had access to only one strain from Brazil) should be analyzed, a high level of mitochondrial similarity (F=0.97) between Libyan and Brazilian strains indicate that some region(s) of South America could very well be the source of the 1988 infestation in Libya. Clearly, additional studies are warranted.

The second goal was to find PCR-based DNA marker to distinguish Libyan or South American flies from those of Mexico, Jamaica and Central American. As shown in Table 4, total DNA amplified by primer pair 5 (Table 1) followed by digestion with *Hpa*I or *Ssp*I produced patterns which can be used to distinguish Libyan and South American strains from those of Mexico, Jamaica and Central America.

The third goal was to determine genetic differences, if any, between the Costa Rica samples collected in 1986 and 1991. Our results (Table 3) showed that COR-91 was polymorphic for *Hind*III and *Sst*III sites, whereas COR-86 was monomorphic (data from Roehrdanz 1989). The COR-91 contained two patterns, D and G for *Hind*III and A and B for *Sst*I, whereas, COR-86 was fixed for pattern A for *Hind*III and pattern A for *Sst*I. In addition, COR-91 was fixed for pattern A for *Msp*I, whereas, COR-86 was fixed for pattern B for *Msp*I. These differences can be attributed to either livestock or human-assisted migrations of flies or simply due to sampling error.

Variation in restriction sites in mtDNA within and among populations are common (reviews by Avise & Lansman 1983, Wilson et al. 1985, Avise 1986, Avise et al. 1987, Moritz et al. 1987). Kessler & Avise (1985) reported significant spatial heterogeneity in the distribution of mtDNA variants within populations (of cotton rats). In addition, mtDNA length variation within an individual (heteroplasmy) and between individuals have been reported in other organisms (reviewed by Moritz et al. 1987). During our studies, except for one individual from COR-91, which produced a mixture of patterns D and G for *Hind*III, we did not detect any evidence of heteroplasmy in populations from South America, Libya, and a Mexican laboratory strain.

The extent of variability observed within and among populations greatly depends upon the analytical power of the method used. For example, the maternally inherited mtDNA and paternally inherited Y-linked genes generally show less variation within populations and more between populations than biparently inherited autosomal nuclear genes (Dowling & Brown 1989). Furthermore, the levels of intra- and inter-species mtDNA RFLP varies greatly among different taxa (Narang et al. 1994). Therefore, it is desirable to use both multiple techniques as well as feral samples of populations to obtain more reliable estimates of genetic variability. Our data provide useful markers to identify geographical populations of screwworm, and should not be regarded as indicators of level of genetic variability of respective geographical populations or for genetic relationships among them.

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References Cited

Avise, J. C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. Roy. Soc. London Ser. B312: 325-342.

AVISE, J. C., J. ARNOLD, R. M. BALL, E. BIRMINGHAM, T. LAMB, J. E. NEIGEL, C. REEB, AND N. C. SAUNDERS. 1987. Intraspecific phylogeny: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol and Syst. 18: 489-522.

AVISE, J. C., AND R. C. LANSMAN. 1983. Polymorphism of mitochondrial DNA in populations of higher animals, pp. 165-190 *in* M. Nei and R. K. Koehn [eds.], *Evolution of Genes and Proteins*. Sinauer, Sunderland, MA.

BOYCE, T. M., M. E. ZWICK, AND C. F. AQUADRO. 1989. Mitochondrial DNA in the bark weevils: size, structure and heteroplasmy. Genetics, 123: 825-836.

DOWLING, T. E., AND W. M. BROWN. 1989. Allozymes, mitochondrial DNA, and levels of phylogenetic resolutions among four species of minnows (Notoropis:Cyprinidae). Syst. Zool. 38: 126-143.

DOWLING, T. E., C. MORITZ, AND J. D. PALMER. 1990. Nuclear Acids II. Restriction site analysis, pp. 250-317 *in* D. M. Hills and C. Moritz [eds.], *Molecular Systematics*. Sinauer, Sunderland, MA.

FAO, 1992. The New World Screwworm Eradication Program. North Africa 1988-1992. pp.192. Food and Agriculture Organization of the United Nations.

HALL, G., AND D. R. SMITH. 1991. Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. Proc. Natl. Acad. Sci. USA 88: 4548-4552.

KESSLER, L. G., AND J. C. AVISE. 1985. Microgeographic lineage analysis by mitochondrial genotype. Variation in the cotton rat (*Sigmodon hispids*). Evolution, 39: 831-838.

KNIPLING, E. F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. J. Econ. Entomol. 48: 459-462.

KNIPLING, E. F., AND H. T. RAINWATER. 1937. Species and incidence of dipteran larvae concerned in wound myiasis. J. Econ. Entomol. 23: 451-455.

LANSMAN, R. A., J. C. AVISE, AND M. D. HUETTEL. 1983. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. Proc. Natl. Acad. Sci. USA 80: 1969-1971.

MORITZ, C., T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: Relevance for population Biology and Systematics. Ann. Rev. Ecol. Syst. 18: 269-292.

NARANG, S. K., W. J. TABACHNICK, AND R. M. FAUST. 1994. Complexities of population genetic structures. Implications for biological control programs, pp. 19-52 *in* Karl Narang, A. C. Bartlett and R. M. Faust [eds.]. *Applications of Genetics to Arthropods of Biological Control Significance*. CRC Press, Boca Raton, FL.

NEI, M., AND W. H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction nuclease. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

REED, K. C., AND D. A. MANN. 1985. Rapid transfer of DNA from agarose gels to nylon membrane. Nucl. Acid Res. 13: 7207-7221.

ROEHRDANZ, R. L. 1989. Intraspecific genetic variability in mitochondrial DNA of the screwworm fly (*Cochliomyia hominivorax*). Biochem. Genet. 27: 551-569.

SIMON, C., A. FRANKE, AND A. MARTIN. 1991. The polymerase chain reaction: DNA extraction and amplification, pp. 329 *in* Hewitt, G. M., A. W. B. Johnson and J. P. W. Young [eds.], *Molecular Taxonomy*, Vol. H57. Nato Advance Study Institute, Springer Verlag, Berlin.

TAYLOR, D. B., L. HAMMACK, AND R. L. ROEHRDANZ. 1991. Reproductive compatibility and mitochondrial DNA restriction site analysis of New World screwworm, *Cochliomyia hominivorax* from North Africa and Central America. Med. Vet. Entomol. 5: 145-151.

WILSON, A. C., L. CANN, S. M. CARR, M. GEORGE, JR., U. B. GYLLENSTEN, K. HELM-BYCHOWSKI, R. C. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, AND M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26: 375-400.