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Mitochondrial DNA variation in screwworm

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Abstract. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to characterize mitochondrial DNA (mtDNA) variation in screwworms, *Cochliomyia hominivorax*, and secondary screwworm, *C. macellaria*, from the Caribbean, North America and South America. Four amplicons, totaling 7.1 kb, were analysed with sixteen restriction enzymes. A total of 133 restriction sites was observed in the two species, 104 in *C. hominivorax*, of which nineteen were variable, and ninety-five in *C. macellaria*, none of which was variable. Fourteen mtDNA haplotypes were observed among eighteen *C. hominivorax* examined. Mean divergence between *C. hominivorax* haplotypes (d) was 0.0064 substitutions per base-pair and genotypic diversity (G) was 0.97. Mean divergence between *C. hominivorax* and *C. macellaria* was 0.0824. *Cochliomyia hominivorax* haplotypes could be divided into three assemblages representing North America, South America and Jamaica, based on UPGMA clustering with d values. The assemblages did not exhibit complete geographic fidelity. These data were discordant with previously published allozyme data indicating little differentiation between screwworm populations. A scenario invoking historically isolated populations coming into contact with the introduction and movement of European livestock is proposed to explain the observed population structure of screwworm.

Key words. *Cochliomyia hominivorax*, *Cochliomyia macellaria*, screwworm, mitochondrial DNA, PCR-RFLP.

Introduction

Screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), is a widespread pest of livestock in the New World tropics. Formerly this species was distributed from the southern United States to northern Argentina and throughout the Caribbean (Guimaraes *et al.*, 1983; Dear, 1985; Rawlins & Mansingh, 1987). Eradication programmes have eliminated screwworm from the United States, Mexico, Guatemala, Belize and El Salvador (Graham, 1985; Vargas-Teran, 1991). Eradication programmes are currently active in the remaining countries of Central America. Population structure and genetic variation in this species have been controversial topics. Richardson and co-workers (Richardson *et al.*, 1982; Richardson & Ellison, 1984) indicated that screwworm was a complex of sibling species or 'gamodemes' in the southwestern United States and Mexico based upon cytological and allozyme differences. Others (LaChance *et al.*, 1982; McInnis, 1983; McInnis *et al.*, 1983) disputed these findings, claiming that screwworm was actually a single polymorphic species. Subsequent research (Dev *et al.*, 1986; LaChance & Whitten, 1986; Krafusur & Whitten, 1993; Taylor &

Peterson, 1994) supports the single species concept. In fact, all of these studies have found screwworm to be a remarkably homogenous species despite its broad geographic range. Dev *et al.* (1986) found no variation in polytene chromosomes of screwworm from the United States, Mexico and the Caribbean. Allozyme studies have found moderate levels of variability, but little differentiation, even between Central and South American populations (Taylor *et al.*, 1996a). Roehrdanz and coworkers (Roehrdanz & Johnson, 1988; Roehrdanz, 1989; Taylor *et al.*, 1991) found screwworm mitochondrial DNA (mtDNA) to be highly variable. Virtually every screwworm sample from southern Mexico had a unique mtDNA haplotype (Roehrdanz, 1989). Less variation was observed in samples from northern Mexico and the United States. However, those samples were obtained from colonies, many of which had been in the laboratory for 5 or more years. Laboratory contamination or bottle-necking could account for the low level of variation observed in the northern populations. Roehrdanz examined few samples from outside of Mexico and no specimens from South America.

Simon *et al.* (1993) compared mtDNA restriction fragment length polymorphism (RFLP) analysis using the whole mtDNA molecule with polymerase chain reaction RFLP (PCR-RFLP) analysis using specific regions of the mtDNA molecule. They determined that the PCR-RFLP technique was faster, less

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expensive, allowed finer resolution, and enabled analysis of poorly preserved samples.

The purpose of this study was to use PCR-RFLP analysis to survey mtDNA variation in screwworms from the Caribbean, Central America and South America. A primary aim of this survey was to determine if phylogeographic correlations can be made such that mtDNA RFLP patterns can be used to determine the geographic origins of unknown screwworm samples. Secondary screwworms, *Cochliomyia macellaria* (F.), from North and South America were examined for outgroup comparisons and to determine the level of mtDNA divergence between the two species.

Materials and Methods

Samples. *Cochliomyia hominivorax* used for this study are listed in Table 1. All samples were stored at -80°C . The Cañas colony was sampled in January 1991 and the Libya colony was sampled in April 1991. The Bijagua, Costa Rica, flies were collected at liver-baited feeding stations (Parker & Welch, 1992). The Dominican Republic and Jamaica samples were from wounds in bovines. The Trinidad collections were from wounds in dogs from Diego Martin, Caguana, Mt Hope and Port of Spain. The samples from Rio Grande do Sul, Brazil, were collected from wounds in bovines near the Universidade Federal Rural do Rio Grande do Sul and reared to adults. No two samples were from the same individual host. Four *C. macellaria*, two from a colony established with flies collected in Fargo, North Dakota (August 1990), and two field collected from wounded calves at the Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil, were analysed.

DNA extraction, amplification, and restriction enzyme analysis. DNA extraction, amplification, restriction digest, and electrophoresis procedures were those used by Taylor *et al.* (1996b). Genomic DNA was isolated using phenol-chloroform extraction and precipitated with ethanol. DNA pellets were dried under vacuum and DNA was resuspended in sterile-distilled water. For amplification, 1 μl of sample DNA was added to a reaction mixture containing 2.5 μl buffer (GeneAmp 10 \times PCR buffer with

15 mM MgCl_2 , Perkin-Elmer, Norwalk, Conn.), 2 μl of dNTP mix (10 mM each: dATP, dTTP, dCTP and dGTP), 1 μl of each primer (20 mM), 1.0 unit of Taq polymerase (Perkin-Elmer) and deionized water to a volume of 25 μl . The PCR profile was thirty-five cycles of 92°C for 1 min, 42°C for 1 min, and 72°C for 2.5 min. Amplicons 1–4 (Fig. 1, Table 2) were examined in all samples and used to detect the mtDNA-RFLP polymorphisms. Based on the *Drosophila yakuba* mtDNA map (Clary & Wolstenholme, 1985) amplicons 1–4 were 2.4, 2.3, 1.6 and 1.3 kb respectively. Amplicons 5–8, as well as the products of S $\text{p}1$ –S $\text{p}3$, S $\text{p}2$ –S $\text{p}4$, C2F3–C3R1, C3F1–N3R2 and N3F1–N3R2, were used to assist in the evaluation of restriction patterns and to partially map restriction sites. Restriction sites in the region of overlap between two amplicons are included in the 5' amplicon of the pair only. Twenty-seven restriction enzymes (RE, *Alu I*, *Apo I*, *Ase I*, *Ava I*, *Ban II*, *Bfa I*, *Bsr I*, *Dde I*, *Dpn II*, *Dra I*, *EcoR I*, *EcoR V*, *Hae III*, *Hinc II*, *Hind III*, *Hinf I*, *Hpa I*, *Mse I*, *Msp I*, *Pvu II*, *Rsa I*, *Sac I*, *Sau96 I*, *ScrF I*, *Ssp I*, *Taq I* and *Xba I*) were screened and sixteen (Table 3) were used for the study.

Vertical polyacrylamide gel electrophoresis (PAGE) was used to analyse the restriction patterns. Digest products were loaded onto 8% gels (16 cm \times 20 cm \times 0.75 mm) in Hoefer (Hoefer Scientific Instruments, San Francisco, Calif.) SE 600 electrophoresis units. Gels were run at constant 300 V (15 mA/gel) for 1.5 h at 20°C , stained for 5 min with ethidium bromide (1 $\mu\text{g}/\text{ml}$) and interpreted using an ultraviolet (312 nm) transilluminator.

A molecular size standard, pGEM[®] DNA Markers (Promega, Madison, Wis.), was included on each gel. Fragment sizes were calculated with GEL-JML (LaCroix, 1994) and extrapolation. Because conformation, as well as size, affects DNA migration rates on polyacrylamide gels, sizes were adjusted based upon the expected length of the amplicon (using the mitochondrial DNA sequence of *Drosophila yakuba* [Clary & Wolstenholme, 1985]), and the position of some of the restriction sites within amplicons 5–8, S $\text{p}1$ –S $\text{p}3$, S $\text{p}2$ –S $\text{p}4$, C2F3–C3R1, C3F1–N3R2 and N3F1–N3R2. Composite haplotypes were determined by combining haplotypes for each of the amplicons.

Statistical analysis. Sequence divergence (*d*) was calculated using The Restriction Enzyme Analysis Package (REAP)

Table 1. *Cochliomyia hominivorax* samples.

| Location | No. of individuals | Type | Collected |
|-------------------------|--------------------|---------------|---------------|
| Costa Rica | | | |
| Bijagua, Alajuela (BJ) | 3 | Field, adults | October 1992 |
| Cañas, Guanacaste | 1 | Colony, adult | 1989 |
| Caribbean | | | |
| Cuba, Guantanamo Bay | 1 | Field, pupae | July 1993 |
| Dominican Republic (DR) | 2 | Field, larvae | October 1992 |
| Jamaica | 1 | Field, larvae | October 1992 |
| Trinidad (TT) | 4 | Field, larvae | November 1992 |
| Brazil | | | |
| Rio Grande do Sul (RGS) | 5 | Field, adult* | March 1993 |
| North Africa | | | |
| Libya | 1 | Colony, adult | October 1990 |

* Collected as larvae from wounds and reared.

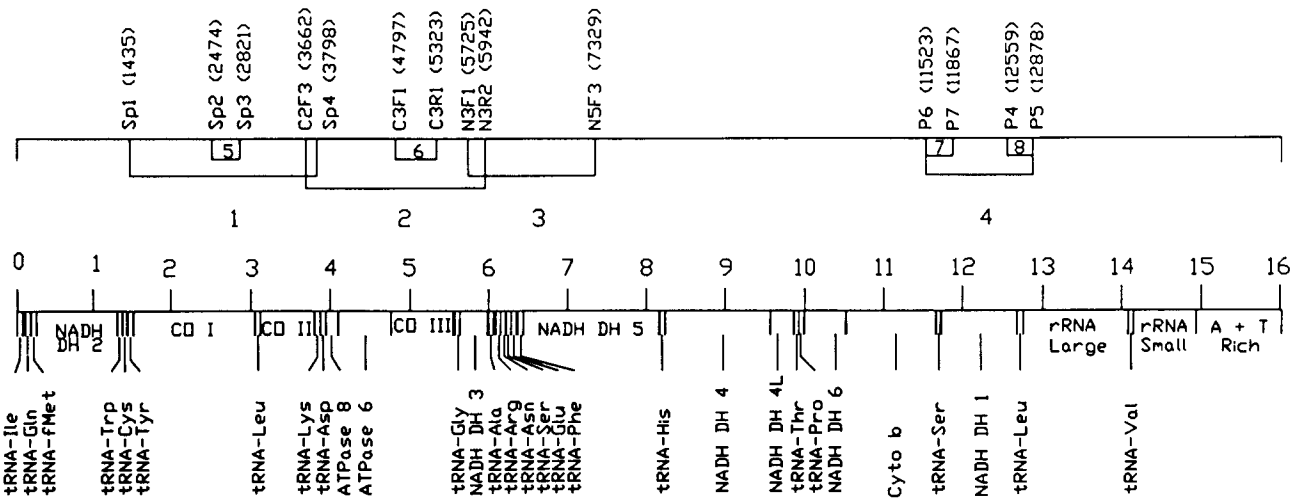


Fig. 1. Mitochondrial DNA molecule of *D. yakuba* (Clary & Wolstenholme, 1985). Genes are indicated below the line and location (bp × 10³) is indicated above the line. The upper line indicates the location of the amplicons used for this study. Numbers in parentheses following the primer names indicate the location of the 5' end of the primer on the *D. yakuba* map.

Table 2. Polymerase chain reaction primers.

| Amplicon | Forward primer | Sequence 5'-3' | Reverse primer | Sequence 5'-3' | Location* |
|----------|-------------------|-----------------------------|-------------------|-----------------------------|-------------|
| 1 | Sp-1 [‡] | TACAATTTATCGCCTAAACTTCAGCC | Sp-4 [‡] | GAGACCATTACTTGCTTTCAGTCATCT | 1435-3793 |
| 2 | C2F3 [§] | GGTCAATGTTTCAGAAATTTGTGG | N3R2 [§] | TGATTTTCATTCATGATATAGTCC | 3662-5942 |
| 3 | N3F1 [§] | CCTTTTGAATGTGGATTTGATCC | N5F3 [§] | TGTCTACTTTAAGACAATTAGG | 5725-7329 |
| 4 | P6 [§] | ACATGAATTGGAGCTCGACCAGT | P5 [§] | GAGTTCAAACCGGCGTAAGCCAGGT | 11523-12878 |
| 5 | Sp-2 [‡] | CAGCTACTTTATGAGCTTTAGG | Sp-3 [‡] | CATTTCAAGCTGTGTAAGCATC | 2474-2821 |
| 6 | C3F1 [§] | ACAGGTGCTATTGGAGCTAT | C3R1 [§] | CCATAAACTGAATCAGCAAT | 4797-5323 |
| 7 | P6 [§] | | P7 [§] | GGTACATTACCTCGGTTTCGTTATGAT | 11523-11867 |
| 8 | P4 [§] | GGTCCCTTACGAATTTGAATATATCCT | P5 [§] | | 12559-12878 |

* *Drosophila yakuba* mtDNA map (Clary & Wolstenholme, 1985). [‡] Sperling *et al.* (1994). [§] Pruess *et al.* (1992). [§] K. Pruess, personal communication.

(McElroy *et al.*, 1992) following the procedures of Nei & Tajima (1981) and Nei & Miller (1990). Genotypic diversity (*G*) was computed by $(n/[n-1])(1-\sum f_i^2)$ where f_i is the frequency of the *i*th mtDNA haplotype in a sample of *n* individuals. *G* is the probability that random matings will be between individuals with different mtDNA haplotypes (Nei, 1987). PHYLIP 3.5 (written by J. Felsenstein) was used to derive a consensus dendrogram of the mtDNA haplotypes using the Wagner parsimony method (MIX and CONSENS programs) and a phenetic tree based upon the UPGMA method using *d* as the distance measure (NEIGHBOR program). *Cochliomyia macellaria* was used as the outgroup.

Results

We examined 7.1 kb representing approximately 45% of the mitochondrial genome (Roehrdanz, 1988). 133 restriction sites were observed in *C. hominivorax* and *C. macellaria*. 104 of the sites were observed in *C. hominivorax*, nineteen of which were variable

(Table 3) and ninety-five sites were observed in *C. macellaria*, none of which was variable. An average of ninety-one restriction sites were observed per *C. hominivorax*, representing 409 bp or 2.6% of the mitochondrial genome. Estimated fragment lengths are presented in Table 4. All variable fragment patterns within *C. hominivorax* could be explained by the gain or loss of a single restriction site except *Dde I* and *Hinc II* in amplicon 1. Two sites differentiated the fragment patterns for each of those digests. Fragment patterns indicative of a change in either of the sites individually were not observed.

Fourteen haplotypes were observed among eighteen *C. hominivorax* examined (Table 5). Average divergence between the haplotypes (*d*) was 0.0064 (*N* = 91, *SD* = 0.0036) (Fig. 2). The frequency distribution of the *d* values was unimodal. Each of the four samples from Costa Rica was unique; three haplotypes were observed among four samples from Trinidad, and four haplotypes among the five samples from Brazil. The sample from Jamaica and one of the samples from Trinidad shared a relatively divergent haplotype. Only one haplotype was observed among four *C. macellaria* examined. The mean divergence

Table 3. Number of restriction enzyme sites (total/variable) in *C.hominivorax* only (SW), *C.macellaria* only (MC), and shared by both species for each amplicon.

| | Amplicon 1 | | | Amplicon 2 | | | Amplicon 3 | | | Amplicon 4 | | |
|-----------------|------------|----|------|------------|----|------|------------|----|------|------------|----|------|
| | SW | MC | Both | SW | MC | Both | SW | MC | Both | SW | MC | Both |
| <i>Ban II</i> | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Bfa I</i> | 0 | 0 | 4 | 0 | 0 | 4 | 2 | 1 | 0 | 2 | 2 | 0 |
| <i>Bsr I</i> | 1 | 1 | 1 | 0 | 0 | 0 | 1/1 | 0 | 0 | 0 | 0 | 0 |
| <i>Dde I</i> | 2/2 | 0 | 3 | 1/1 | 2 | 1 | 0 | 0 | 2 | 3/1 | 1 | 0 |
| <i>Dpn II</i> | 0/1 | 2 | 6 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 0 |
| <i>Dra I</i> | 2 | 3 | 0 | 1/1 | 0 | 1 | 0 | 0 | 4 | 1 | 1 | 0 |
| <i>EcoRI</i> | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| <i>EcoRV</i> | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hae III</i> | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Hinc II</i> | 2/2 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| <i>Hind III</i> | 0 | 0 | 0 | 0/1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hinf I</i> | 1 | 2 | 2 | 4 | 1 | 2 | 1/1 | 0 | 1 | 1 | 0 | 2 |
| <i>Msp I</i> | 2/1 | 0 | 2 | 1/1 | 0 | 0 | 1/1 | 0 | 1 | 1/1 | 0 | 0 |
| <i>Pvu II</i> | 0/1 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Rsa I</i> | 1 | 1 | 1 | 1/1 | 1 | 4 | 0 | 1 | 0 | 0/1 | 0 | 3 |
| <i>ScrF I</i> | 0 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 1/1 | 0 | 0 |
| Total | 11/7 | 10 | 30 | 11/5 | 10 | 19 | 6/3 | 3 | 11 | 10/4 | 6 | 6 |

Table 4. Estimated restriction fragment lengths (kb) for *C.hominivorax*. Numbers inside parentheses indicate the size of the fragments when a variable site was present. A fragment equal to the sum of the two numbers was present when the variable site was absent.

| Restriction enzyme | Amplicon 1 | Amplicon 2 | Amplicon 3 | Amplicon 4 |
|--------------------|--|--------------------------------------|--------------------|--------------------------------|
| <i>Ban II</i> | 1.46, 0.60, 0.30 | 2.28 | 1.60 | 1.36 |
| <i>Bfa I</i> | 1.00, 0.42, 0.36, 0.34, 0.24 | 0.88, 0.52, 0.32, 0.29, 0.26 | 1.16, 0.29, 0.15 | 0.54, 0.46, 0.36 |
| <i>Bsr I</i> | 0.87, 0.56, 0.40, 0.39, 0.15 | 2.28 | (1.25, 0.35) | 1.36 |
| <i>Dde I</i> | (0.52, 0.41), (0.88, 0.13), 0.41, 0.03 | 1.23, (0.72, 0.33) | 0.95, 0.39, 0.25 | (0.50, 0.41), 0.40 |
| <i>Dpn II</i> | 0.84, 0.67, (0.35, 0.09), 0.15, 0.14, 0.12 | 1.68, 0.60 | 0.75, 0.62, 0.23 | 1.36 |
| <i>Dra I</i> | 1.20, 0.68, 0.48 | (1.12, 0.61), 0.55 | | 1.08, 0.23, 0.04 |
| <i>EcoRI</i> | 2.36 | 1.61, 0.67 | 0.85, 0.75 | 1.01, 0.34 |
| <i>EcoRV</i> | 1.19, 1.11 | 1.75, 0.53 | 1.60 | 1.36 |
| <i>Hae III</i> | 2.36 | 1.13, 0.71, 0.44 | 1.60 | 1.00, 0.35 |
| <i>Hinc II</i> | (0.46, 0.47), (0.16, 0.56), 0.71 | 1.20, 1.08 | 0.87, 0.75 | 1.36 |
| <i>Hind III</i> | 2.36 | 1.61, (0.42, 0.25) | 1.60 | 1.36 |
| <i>Hinf I</i> | 1.16, 0.39, 0.39, 0.33, 0.10 | 1.00, 0.47, 0.42, 0.18, 0.17 | (0.74, 0.50), 0.36 | 0.53, 0.52, 0.30 |
| <i>Msp I</i> | 1.54, (0.32, 0.16), 0.18, 0.15 | (1.66, 0.62) | (1.35, 0.13), 0.12 | (1.02, 0.33) |
| <i>Pvu II</i> | 1.63, (0.51, 0.22) | 1.53, 0.75 | 1.60 | 1.36 |
| <i>Rsa I</i> | 1.53, 0.63, 0.19 | 1.48, (0.22, 0.12), 0.22, 0.13, 0.11 | 1.60 | (0.48, 0.35), 0.34, 0.13, 0.06 |
| <i>ScrF I</i> | 1.40, 0.61, 0.14, 0.14, 0.04 | 1.42, 0.86 | 1.60 | (1.02, 0.33) |

between *C.macellaria* and *C.hominivorax* was 0.0824 ($N = 14$, $SD = 0.0020$). Genotypic diversity, G , for *C.hominivorax* was 0.97.

The parsimony analysis found fifty-eight equally parsimonious trees (length = 26) for the fourteen haplotypes with *C.macellaria* as the outgroup. The consensus tree is presented in Fig. 3. The samples from Costa Rica (excluding Bijagua 2) and Cuba represented a single branch of the cladogram defined by three apomorphic restriction sites (one *Dde I* and two *Hinc II* sites, all in amplicon 1). Bootstrap analysis indicated that this was the only significant node in the cladogram (present in 90% of the

trees from 100 bootstrap replicates). Bijagua 2 did not cluster with the other Costa Rica samples in either the consensus cladogram (Fig. 3) or the phenetic tree (Fig. 2). Rio Grande do Sul and Trinidad samples were distributed throughout both trees. Samples from the northern Caribbean (Cuba, Dominican Republic and Jamaica) failed to cluster as well. The sample from Libya was most closely associated with samples from Brazil and Trinidad. The primary difference between the parsimony and phenetic trees was the position of the Jamaica-TT3 haplotype. The parsimony analysis included this haplotype with the South American and Caribbean haplotypes while the phenetic tree

Table 5. Mitochondrial DNA haplotypes observed in *C.hominivorax* and *C.macellaria*. Sites fixed in *C.hominivorax* but differentiating *C.hominivorax* from *C.macellaria* are not included. A '1' indicates the site was present; a '0' indicates the site was absent.

| Specimen: Site No.: | Amplicon 1 | | | | | | | Amplicon 2 | | | | | Amplicon 3 | | | Amplicon 4 | | | | Haplotype |
|------------------------|-------------------|-------------------|--------------------|---------------------|---------------------|-------------------|--------------------|-------------------|-------------------|-----------------------|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|--------------------|--------------------|---------------------|-----------|
| | <i>Dde I</i> 1 | <i>Dde I</i> 2 | <i>Dpn II</i> 3 | <i>Hinc II</i> 4 | <i>Hinc II</i> 5 | <i>Msp I</i> 6 | <i>Pvu II</i> 7 | <i>Dde I</i> 8 | <i>Dra I</i> 9 | <i>Hind III</i> 10 | <i>Msp I</i> 11 | <i>Rsa I</i> 12 | <i>Bsr I</i> 13 | <i>Hinf I</i> 14 | <i>Msp I</i> 15 | <i>Dde I</i> 16 | <i>Msp I</i> 17 | <i>Rsa I</i> 18 | <i>ScrF I</i> 19 | |
| <i>C.hominivorax</i> | | | | | | | | | | | | | | | | | | | | |
| Costa Rica | | | | | | | | | | | | | | | | | | | | |
| BJ 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| BJ 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| BJ 3 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 3 |
| Cañas | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| Caribbean | | | | | | | | | | | | | | | | | | | | |
| Cuba | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 5 |
| DR 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| DR 2 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Jamaica | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 7 |
| TT 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 8 |
| TT 2 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 8 |
| TT 3 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 7 |
| TT 4 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 9 |
| Brazil | | | | | | | | | | | | | | | | | | | | |
| RGS 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 10 |
| RGS 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| RGS 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| RGS 4 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| RGS 5 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 13 |
| North Africa | | | | | | | | | | | | | | | | | | | | |
| Libya | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 14 |
| <i>C.macellaria</i> | | | | | | | | | | | | | | | | | | | | |
| Fargo | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 15 |
| Rio Janeiro | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 15 |

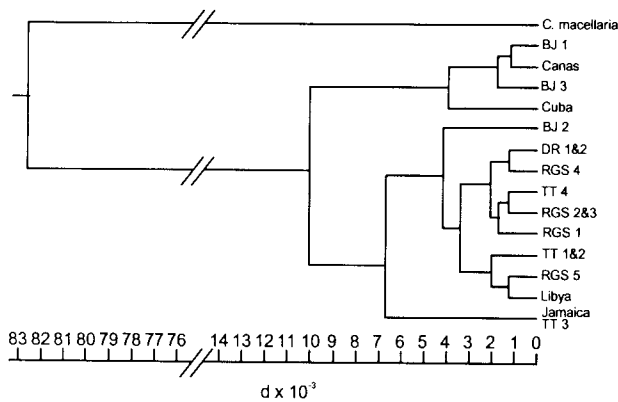


Fig. 2. Phenetic tree of *C. hominivorax* and *C. macellaria* populations using *d* as the distance measure. The phenogram was constructed using the UPGMA method.

segregated this haplotype out. The phenetic tree indicates three assemblages of haplotypes, roughly based upon Costa Rica (excluding Bijagua 2 and including Cuba), Jamaica (including

Trinidad 3) and South America (the remaining samples). Mean divergence among the assemblages was 0.009 (SD = 0.0024, *N* = 50) compared with 0.003 (SD = 0.0013, *N* = 41) within the assemblages.

Discussion

Cochliomyia hominivorax is a highly variable species with respect to mtDNA. We observed fourteen haplotypes among the eighteen samples examined. Variation was high even within local populations. Limited phylogeographic structure was observed among screwworm populations. Combining our data for the Caribbean, Central America and South America with those of Roehrdanz (1989) for Mexico strengthens the Costa Rica branch of the cladogram. The two *Hinc II* sites in amplicon I were absent from South American and most of the Caribbean samples. Roehrdanz found one of these sites (= *Hpa I*) present in nearly all of his samples from Mexico and Texas (by using *Hpa I* Roehrdanz was not able to detect the second *Hinc II* site). Therefore these sites appear to be characteristic of North American screwworms. Costa Rica may be in the contact zone between the North American

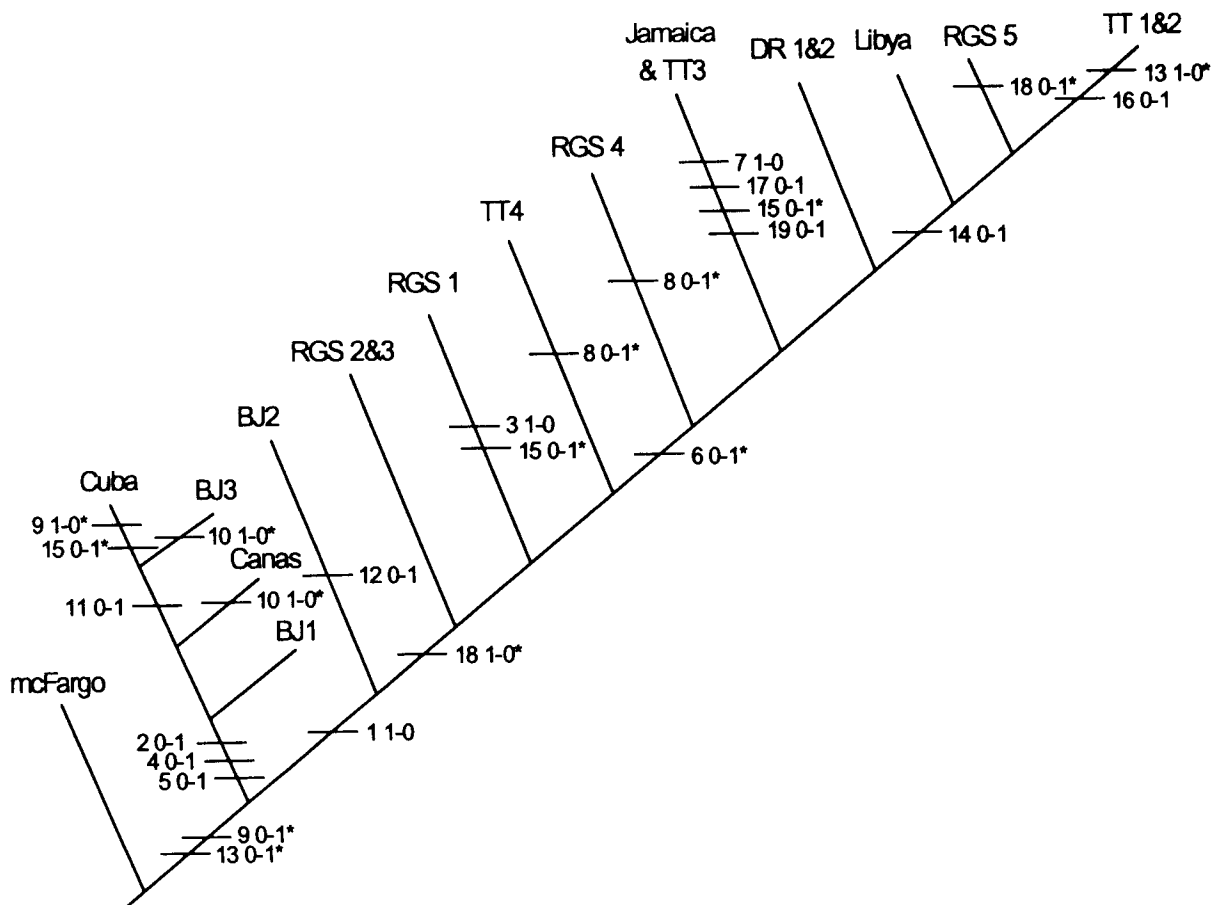


Fig. 3. Consensus Wagner Parsimony tree for *C. hominivorax* and *C. macellaria*. Transition series and character state changes are indicated by line breaks. Site numbers are from Table 5. Site changes denoted by asterisks occur more than once in the tree.

and South American populations, accounting for Bijagua-2 which was more closely associated with the South American samples. The *Hind III* site in amplicon 2 showed a similar pattern. This site was present in all of our South American and Caribbean samples, but was absent in all of Roehrdanz's (1989) North American samples except two. The *Dde I* sites in amplicon 2 appear to be associated with the *Hinc II* sites and may follow the same pattern. As in Roehrdanz's study, we observed a divergent haplotype in the sample from Jamaica. Our Jamaica matched that of Roehrdanz for all of the common sites except *Hind III* in amplicon 2. We found this site to be present in Jamaica and Roehrdanz found it to be absent. This haplotype was also observed in one of the samples from Trinidad.

Based upon the phenetic analysis, screwworm appears to consist of three discontinuous assemblages of mitochondrial haplotypes: (1) North and Central America, (2) Jamaica, and (3) South America. This pattern fits category I of Avise's phylogeographic model (Avise *et al.*, 1987). Category I, characterized by phylogenetic discontinuities with spatial separation, is the most commonly encountered. Crossing studies between screwworms from each of the three haplotype assemblages have found no evidence of pre- or post-copulatory isolation under laboratory conditions (Taylor *et al.*, 1991; C. J. Whitten, personal communication). Furthermore, sterile flies, predominately of the North American haplotype, successfully eradicated screwworm from Libya which were of the South American assemblage (Vargas-Teran *et al.*, 1994).

In contrast with *C.hominivorax*, mtDNA variation in *C.macellaria* appears to be limited. Although our sample size was small, the geographic distance between the samples was large. We also failed to find mtDNA variation in *C.macellaria* while examining five, 100–350 bp, amplicons in flies from North America, Central America and South America (Taylor *et al.*, 1996b). In that study, five mtDNA haplotypes were detected among eight *C.hominivorax* from a single colony originating from Costa Rica. Avise *et al.* (1984) present an argument that mtDNA diversity is related to population size in species with continuous population structure. Allozyme data (Taylor & Peterson, 1994, 1995) indicate that *C.macellaria* has a continuous population structure. Genetic distances among populations from North America, Central America and South America were ≤ 0.002 . Populations of *C.macellaria* are much higher than those of *C.hominivorax*; estimates range from 10:1 at Swormlure baited traps (Mackley, 1986) to 590:1 at wounds, and 2427:1 at meat-baited fly traps (Laake *et al.*, 1936). Consequently, one would expect *C.macellaria* to have proportionally higher mtDNA diversity relative to *C.hominivorax*. The high level of mtDNA variability despite the low population levels of *C.hominivorax* indicates that screwworm populations may have been discontinuous historically.

The nucleotide substitution rate for invertebrate mtDNA is estimated to be approximately 2% per million years (my), ± 0.5 my (Powell *et al.*, 1986). Based upon this estimate, *C.hominivorax* and *C.macellaria* diverged 4–12 my ago. This estimate is lower than the estimate of 17–24 my based on allozyme data (Taylor & Peterson, 1994). Time of divergence for the *C.hominivorax* haplotype assemblages is estimated to be 0.55–1.65 my for North and South America, 0.4–1.2 my for South American and Jamaica, and 0.75–2.25 my for North America and Jamaica. The primary

interchange of North and South American land faunas occurred 1.5–2.5 my ago (Webb, 1985). This correlates well with the level of divergence observed between North and South American haplotype assemblages assuming *C.hominivorax* crossed the Panamanian corridor to colonize both continents. More samples are needed from the Caribbean region before we can speculate on the biogeographical meaning of the Jamaican haplotype assemblage.

Despite the apparent genetic discontinuity of screwworm populations indicated by mtDNA, allozyme data indicate high levels of gene flow between screwworm populations (Taylor & Peterson, 1994; Taylor *et al.*, 1996a). Genetic distances between Central American and South American populations were ≥ 0.006 . Two possible explanations for the discordance between allozyme and mtDNA data are (1) gene flow has been maintained between populations by males or, more likely, (2) the introduction and movement of livestock during the last 500 years have augmented gene flow between previously isolated populations. Screwworm dependence on wounds in warm-blooded vertebrates may have limited its distribution to pockets where adequate hosts were available. The introduction and movement of cattle and other livestock by European colonizers increased the number of potential hosts and may have provided the bridges necessary to permit the gene flow indicated by the allozyme data. *Cochliomyia macellaria* uses carrion, a less restrictive habitat, for larval development and has been able to maintain widespread, continuous populations. Recent, human mediated dispersal of screwworm is supported by the observation of rare, divergent, haplotypes outside of their proposed range. Roehrdanz (1989) found one such sample, STD32, from Quintana Roo, Mexico, which was more similar to the Jamaican haplotype than that of the other Mexican samples. We observed two samples, Bijagua 2 and Trinidad 3, which were more similar to geographically distant populations than their local counterparts.

Because our sample sizes from the northern Caribbean were small, they can be used only to indicate the presence of the observed haplotypes and provide no information concerning the absence of other haplotypes. Given this limitation, they still raise several questions. The lone sample from Cuba appears to be allied with the North American haplotypes, whereas both samples from the Dominican Republic are more similar to the South American haplotypes and Jamaica has its own divergent haplotype. This would indicate that the screwworm populations in the northern Caribbean are of multiple origins. The Jamaican haplotypes are most interesting. Three samples collected over a 15-year period have had similar haplotypes. This indicates that this haplotype assemblage predominates on Jamaica. This raises the question as to whether or not this assemblage is endemic to Jamaica, and each of the Caribbean islands historically had endemic screwworm populations, or is this a relict haplotype which was found throughout the Caribbean before the introduction of North and South American haplotypes. More samples from the Caribbean will need to be analysed to answer this question.

One aim of this study was to determine if mtDNA characteristics can be used to determine the origin of screwworm samples. The PCR-RFLP technique appears to have the potential to differentiate between samples from North and South America within reasonable limits of certainty. However, most of the North American populations have been eradicated. Screwworm

populations in Central America, at least from Costa Rica, appear to be a mixture of North and South American haplotypes. The lack of phylogeographic structure among the samples from Trinidad and Brazil suggests little mtDNA differentiation in South America and variation in samples from the Caribbean indicates multiple origins for screwworms from that region. The presence of the Jamaican haplotypes in Quintana Roo, Mexico and Trinidad precludes the possibility of assigning the origin of samples with this divergent haplotype. As an example, the origin of the 1988 screwworm outbreak in Libya has been the focus of much attention. Taylor *et al.* (1991) indicated that the outbreak probably originated from somewhere outside of North or Central America. Beesley (1991) and Narang & Degrugillier (1995) indicate the outbreak probably originated from South America. Our results are concurrent with those conclusions. The Libya haplotype is part of the South American assemblage supporting the South American origin of the introduction. However, flies with South America haplotypes were observed from Dominican Republic as well, indicating that the Caribbean cannot be eliminated as the origin of the outbreak.

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