Journal of the American Mosquito Control Association, 16(2):171–174, 2000
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SCIENTIFIC NOTE

CORRELATED MORPHOLOGIC AND GENETIC DIVERSITY AMONG *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) COLLECTIONS IN VENEZUELA

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ABSTRACT. Diversity among *Lutzomyia longipalpis* populations in Venezuela was characterized using 2 methods: larval mouthpart morphology–morphometry and isoenzyme electrophoresis. Analysis of the results suggested the presence of 2 morpho-genotypes. The mentum, maxillary comb, mandibular ventral teeth, and adenylate kinase and hexokinase enzyme-encoding loci suggested that a population from the northwestern Coriano System (Curarigua) is a distinct lineage within the *L. longipalpis* complex. Three widely separated populations from the Llanos (savanna), Andes, and northcentral Coastal Cordillera showed no significant substructure. These studies provide morphologic markers that are congruent with genetic data and suggest that the morphologic markers may be used to characterize and differentiate populations within this species complex.

KEY WORDS isoenzyme, larval mouthpart, *Lutzomyia longipalpis*, visceral leishmaniasis

Lutzomyia longipalpis (Lutz and Neiva) is the principal vector of New World visceral leishmaniasis and is widespread in Mexico and Central and South America (Young and Duncan 1994). Extensive biological variability in this vector has been revealed in studies of morphology, pheromones, behavior, isoenzymes, and the *maxadilan* gene (Mangabeira 1969, Ward 1986, Ward et al. 1988, Lanzaro et al. 1993, Warburg et al. 1994, Morrison et al. 1995, Azevedo et al. 1997, Dujardin et al. 1997, Mukhopadhyay et al. 1998, Mutebi et al. 1998). Some of these authors suggest that *L. longipalpis* is a complex composed of at least 2 or 3 species. That observation has been based on genetic (isoenzymes) or phenotype (pheromone type) data combined in some cases with cross-mating experiments (Ward et al. 1988; Lanzaro et al. 1993; Hamilton et al. 1996a, 1996b; Hearne et al. 1998).

Attempts to show correlations between phenotypic and genetic characters largely have been unsuccessful. Ward et al. (1988) reported no correlation between the number of tergal pale spots in males, pheromone type, and genetic data among populations (Ward 1986). Lanzaro et al. (1993) showed divergence among 3 populations of *L. longipalpis* by isoenzymes. However, these populations were found to share the same pheromone type (Hamilton et al. 1996a, 1996b; Hearne et al. 1998).

Mutebi et al. (1998) found genetic homogeneity among *L. longipalpis* populations incongruent with pheromone heterogeneity reported by Hamilton et al. (1996c) for Central American populations. However, Mukhopadhyay et al. (1998) suggested that the Jacobina population from Brazil was differentiated from other Brazilian populations based upon heterogeneity in allozyme frequencies, pheromone type, and behavioral data (Hamilton and Ward 1991; Hamilton and Ramsoondar 1994; Hamilton et al. 1996a, 1996b). However, these authors did not discuss the close relationship between the Lapinha/Callejon and Santarem/Marajo populations that produce very different pheromone types (Harris et al. 1998). Dujardin et al. (1997) were the 1st to report a correlation between wing morphometry and isoenzyme markers among Bolivian populations of *L. longipalpis*.

The purpose of this note is to report congruence between genetic variation at isoenzyme loci and larval morphology. The morphology of larval mouthparts previously never has been used to explore differentiation among sandfly populations, specifically within a species complex. These characters have been examined extensively in Venezuelan populations that have also been analyzed during epidemiologic and ecological studies. A Curarigua population has been studied under other criteria as well including random amplified polymorphic DNA–polymerase chain reaction markers, insecticide resistance, and pheromone characteristics (Arrivillaga et al. 1995, 1996; Mazzarri et al. 1997; Hearne et al. 1998).

Four hundred *L.* (*Lutzomyia*) *longipalpis* adults were collected in Curarigua, Lara State (9°59′N, 69°55′W); Cojedes, Cojedes State (9°42′N, 68° 26′W); Guayabita, Aragua State (10°16′N, 67° 28′W); and Trujillo, Trujillo State (9°25′N, 70°30′W) (Fig. 1). Isozymes were analyzed in these adults. We included *L.* (*Lutzomyia*) *gomezi* (Nitzulescu) as an outgroup. Both *L. longipalpis* (series Longipalpis)

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Fig. 1. Collection sites of *Lutzomyia longipalpis* in Venezuela.

and *L. gomezi* (series Cruciata) species are morphologically distinct and are considered in the current classification to belong to the subgenus *Lutzomyia* (Young and Duncan 1994).

One hundred F_1 4th-stage larvae reared from field-collected adult females (Curarigua, Cojedes, and Trujillo) were also characterized by morphologic and morphometric analyses. *Lutzomyia longipalpis* populations from Colombia and Brazil (Lapinha and Jacobina) were also included. In the morphologic comparison *L. gomezi, L. youngi* (Verrucarum group) (Feliciangeli and Murillo), and *Phlebotomus papatasi* (Scopoli) (from Italy) were included. The outgroup (*Phlebotomus papatasi*) and reference taxa were used to root the trees without the intention of exploring the sister relationships of *L. longipalpis*.

Polyacrylamide gel electrophoresis was performed on individual *L. longipalpis* (adults and larvae) following the protocol of Munstermann (1980). We examined flies from these populations using 16 enzyme loci available in our laboratory. No activity was observed for 3 enzymes (glucose dehydrogenase, EC 1.1.1.118.; D-2-hydroxy-acid dehydrogenase, EC 1.1.99.6; and glutamic-oxaloacetic transaminase EC 2.6.1.1.). Five enzymes

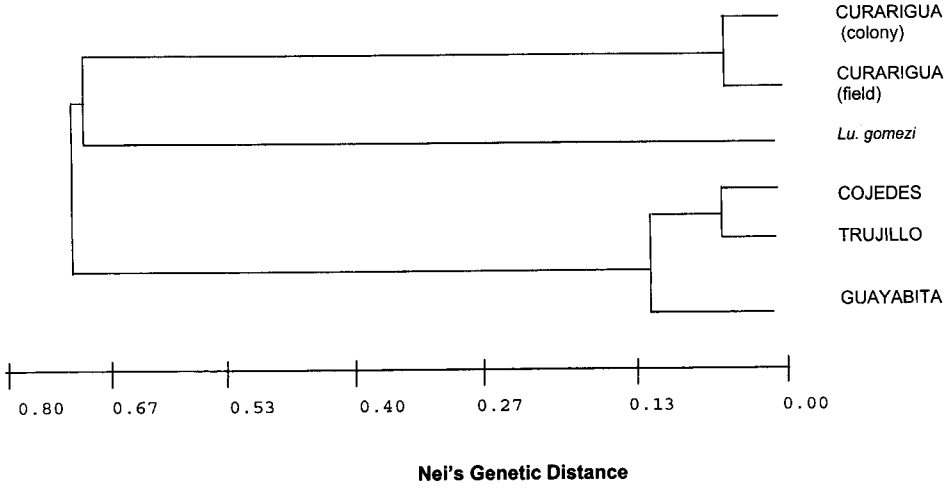

Fig. 2. UPGMA dendogram based on Nei's genetic distance among samples of *Lutzomyia longipalpis* from Venezuela.

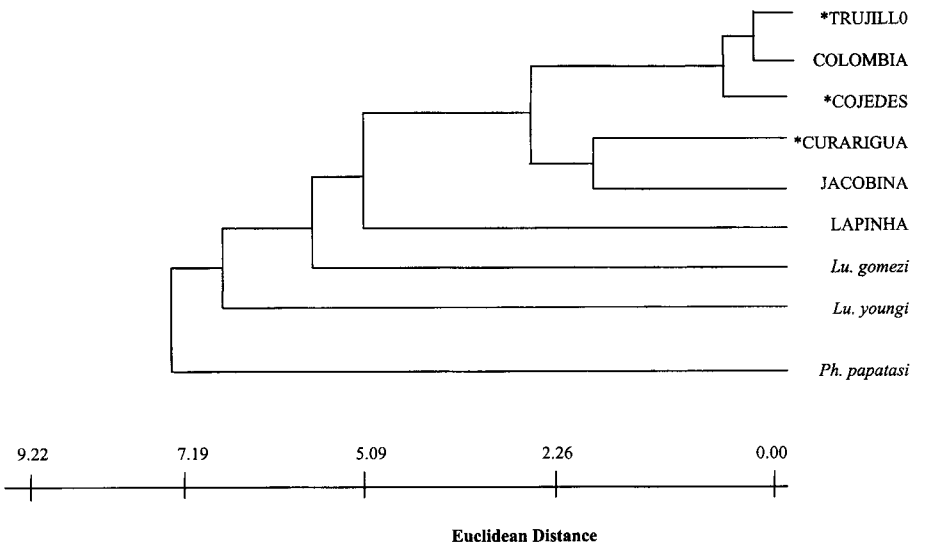

Fig. 3. UPGMA dendogram based on Euclidean distance among several sandflies species by 26 larval mouthpart characters (* = Venezuelan samples).

(fumarase, EC 4.2.1.2.; esterase, EC 3.1.1. . ., α-glycerophosphate dehydrogenase EC 1.1.1.8.; phosphoglucomutase, EC 5.4.2.2.; and lactic acid dehydrogenase EC 1.1.1.27.) did not demonstrate clear resolution of bands and were not scorable on gels. Aldehyde oxidase (EC 1.2.3.1.) showed variable patterns among individuals that were not amenable to allelic interpretation. Genetic variability was successfully scored at 7 enzyme loci: Ak (adenylate kinase, EC 2.7.4.3.), Ark (arginine kinase, EC 2.7.3.3.), Gpi (glucosephosphate isomerase, EC 5.3.1.9), Hk (hexokinase, EC 2.7.1.1.), Idh (isocitrate dehydrogenase, EC 1.1.1.42), Me (malic enzyme, EC 1.1.1.40), and Mdh-2 (malate dehydrogenase, EC 1.1.1.37). Genetic variability statistics were estimated using BIOSYS-1 (Swofford and Selander 1981). Genotype frequencies were analyzed for compliance with Hardy–Weinberg expectations and genetic relatedness measures were estimated among collections with the same software.

Twenty-six characters were compared among 6 *L. longipalpis* collections. These characters were based on a previous redescription of larval mouthpart morphology and the mouthpart nomenclature for the *Lutzomyia* genus (Arrivillaga et al. 1999a, 1996b). All of the 26 morphometric characters were analyzed using Euclidean distance and cluster analysis (unweighted pair-group method using arithmetic averages [UPGMA]). The characters were also analyzed by principal component analysis using the MVSP 2.1 program (Kovach 1993). The qualitative analysis was based on larval mouthpart phenotypes.

Patterns of morphometric and isoenzyme variations were concordant among the Venezuelan *L. longipalpis* populations and provided evidence of structuring among populations. Patterns were consistent with a hypothesis of 2 morpho-genotypes in Venezuela. The Curarigua (northwestern) collection is a distinct lineage (Figs. 2 and 3) identified by the diagnostic enzyme loci Ak and Hk. Variance in allozyme frequencies suggest restricted gene flow ($Nm < 1$) between Curarigua and the other populations analyzed. The populations from Guayabita (north-central Coastal Cordillera), Cojedes (Central Llanos), and Trujillo (Andes Cordillera) showed some substructuring. We currently are examining the distribution of mtDNA haplotypes within populations of *L. longipalpis* from Venezuela and among other reference populations of non-Venezuelan *L. longipalpis*. Preliminary phylogenetic analyses support the morphologic and genetic differentiation among Venezuelan populations (Arrivillaga et al., in preparation).

Phenotypic characters were typically mentum and mandibular ventral teeth morphology and morphometry and maxillary comb morphology. Our morphologic results (Fig. 3) support a hypothesis of at least 3 *L. longipalpis* morphotypes. The differentiations between Brazilian and Colombian populations and between Lapinha and Jacobina (Brazilian) populations are congruent with the genetic differentiation reported by Lanzaro et al. (1993), Morrison et al. (1995), and Mukhopadhyay et al. (1998). These results support the use of morphologic criteria based on larval characters to identify sibling species in the *L. longipalpis* complex and suggest that their utility should be explored in other sand fly groups.

This work was supported by Venezuela Gov-World Bank (project PCEE-VEN96-002-006) and the Conicit-Becas Venezuela. We especially thank Arturo Bravo, Maria Martinez, Florencio Mendoza, and Hermes Piñago for technical assistance, B.

Tesh and G. Lanzaro for comments and suggestions, and M. Maroli for providing the samples of *P. papatasi*.

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