AD-A270 191

AA 078 43

ATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE

Lutzomyia Longipalpis is a Species Complex:Genetic Divergence and Interspecific Hybrid Sterility Among Three Populations.

5. FUNDING NUMBERS

6. AUTHOR(S)

Gregory C. Lanzaro, Katherine Ostrovska, Phillip G. Lawyer Marco V. Herrero, and Alon Warburg

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Walter Reed Army Institute of Research
Washington, DC 20307-5100

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research & Development Command
Ft. Detrick, Frederick, MD 21701-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

NTIS CR
DTIC TA
Unannou
Justificati

11. SUPPLEMENTARY NOTES

By
Distribution/

12a. DISTRIBUTION/AVAILABILITY STATEMENT

APPROVED FOR PUBLIC RELEASE:
DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

Dist	Availability or Special
A-1	20

13. ABSTRACT (Maximum 200 words)

The sand fly Lutzomyia longipalpis is the vector of Leishmania donovani chagasi in Latin America. An analysis of genetic variability at 27 enzyme coding loci among three laboratory populations of Lu. longipalpis revealed substantial genetic polymorphism. Levels of genetic distance between all pairwise comparisons of colonies were very high, and consistent with those previously reported among separate species in the genus Lutzomyia. Between 7% and 22% of the loci studied were diagnostic for any two of the colony populations. Experimental hybridization between colonies resulted in the production of sexually sterile male progeny. Our results provide strong evidence that Lu. longipalpis exists in nature as a complex of at least three distinct species. The possible effects of colonization on the genetic makeup of laboratory populations is considered in extending our results to natural populations.

14. SUBJECT TERMS

Lutzomyia longipalpis, Leishmania donovani chagasi

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

NSN 7540-01-280-5500

Am. J. Trop. Med. Hyg., 48(6), 1993, pp. 839–847

LUTZOMYIA LONGIPALPIS IS A SPECIES COMPLEX: GENETIC DIVERGENCE AND INTERSPECIFIC HYBRID STERILITY AMONG THREE POPULATIONS

GREGORY C. LANZARO, KATHERINE OSTROVSKA, MARCO V. HERRERO, PHILLIP G. LAWYER, AND ALON WARBURG

Department of Entomology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, District of Columbia; Laboratory of Malaria Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; Tropical Disease Research Program, School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica

Abstract. The sand fly *Lutzomyia longipalpis* is the vector of *Leishmania donovani chagasi* in Latin America. An analysis of genetic variability at 27 enzyme coding loci among three laboratory populations of *Lu. longipalpis* revealed substantial genetic polymorphism. Levels of genetic distance between all pairwise comparisons of colonies were very high, and consistent with those previously reported among separate species in the genus *Lutzomyia*. Between 7% and 22% of the loci studied were diagnostic for any two of the colony populations. Experimental hybridization between colonies resulted in the production of sexually sterile male progeny. Our results provide strong evidence that *Lu. longipalpis* exists in nature as a complex of at least three distinct species. The possible effects of colonization on the genetic makeup of laboratory populations is considered in extending our results to natural populations.

Visceral leishmaniasis is a potentially fatal disease affecting large populations in many parts of the world. In Latin America alone, some 1.6 million people are considered at risk, and almost 200,000 cases occur annually.[1] The causative agent is *Leishmania donovani chagasi*, which is transmitted by the Phlebotomine sand fly *Lutzomyia longipalpis*. Dense populations of *Lu. longipalpis* are frequently associated with human habitation in many of the drier regions of Latin America. The species has been found in all known foci of visceral leishmaniasis and is a proven vector in some of them.[2–5] More recently, *L. d. chagasi* has also been isolated from persons with a nonulcerative form of cutaneous leishmaniasis in Central America.[6,7] *Lutzomyia longipalpis* was the predominant anthropophilic sand fly in these foci as well.

The geographic distribution of *Lu. longipalpis* extends from Mexico to southern Brazil and consequently covers a variety of habitats. This large range is subdivided by mountain ranges and zones of unsuitable climate that apparently pose formidable barriers to migration. Phlebotimine sand flies are generally poor fliers with movement restricted to short, flight-assisted hopping. Flight ranges for several neotropical species in the genus *Lutzomyia* have been estimated not to exceed 100 meters in a 24-hr period.[8] Wind-assisted dispersal has been shown not to be significant.[9] Geographic isolation among populations of a species with low vagility can promote genetic divergence resulting from genetic drift and/or selection for adaptation to regional habitats. Eventually postzygotic or prezygotic reproductive isolating mechanisms (RIM) may evolve. If reproductive isolation occurs, such populations become independent gene pools, each representing a species that follows a distinct evolutionary trajectory. Ward and others have suggested that *Lu. longipalpis* may be a species complex.[10] Their conclusions were based on a series of attempted matings between populations that differed in the number of pale spots on the abdomens of males (one pair of spots versus two pairs). They did not find sperm in female spermathecae and suggested the existence of pre-RIM. In the few cases in which hybrids were produced, both sexes were sexually fertile. Although their results are interesting, the failure of mating in caged populations cannot be taken as proof of pre-RIM in nature. A study of the genetic structure of a single population of *Lu. longipalpis* from Bolivia, consisting of a mixture of individuals that varied in body size, revealed no evidence of significant genetic divergence among different body size

TABLE 1

*Enzyme loci studied**

Enzyme	E.C. number†	Abbreviation	Buffer‡
Aconitase-1	4.2.1.3	ACON-1	CA-8
Aconitase-2	4.2.1.3	ACON-2	CA-8
Adenylate kinase	2.7.4.3	ADK	C
Aldolase	4.1.2.13	ALD	C
Aldehyde oxidase-1	1.2.3.1	AO-1	CA-8
Aldehyde oxidase-2	1.2.3.1	AO-2	CA-8
Diaphorase	1.6.2.2	DIA	C
Fumarase	4.2.1.2	FUM	CA-8
α-glycerophosphate dehydrogenase	1.1.1.8	α-GDH	CA-8
Glutamate oxaloacetate transaminase-1	2.6.1.1	GOT-1	CA-8
Glutamate oxaloacetate transaminase-2	2.6.1.1	GOT-2	CA-8
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	G3PDH	CA-7
Hydroxyacid dehydrogenase	1.1.1.30	HAD	CA-5.5
Hexokinase	2.7.1.1	HK	TBE
Isocitrate dehydrogenase-1	1.1.1.42	IDH-1	CA-8
Isocitrate dehydrogenase-2	1.1.1.42	IDH-2	CA-8
Malic acid dehydrogenase-1	1.1.1.37	MDH-1	CA-7
Malic acid dehydrogenase-2	1.1.1.37	MDH-2	CA-7
Malic enzyme-1	1.1.1.40	ME-1	CA-7
Malic enzyme-2	1.1.1.40	ME-2	TBE
Mannose-6-phosphate isomerase	5.3.1.8	MPI	CA-7
Peptidase-1	3.4.1.1	PEP-1	CA-8
Peptidase-2	3.4.1.1	PEP-2	CA-8
6-Phosphogluconic acid dehydrogenase	1.1.1.44	6-PGD	CA-7
Phosphoglucoisomerase	5.3.1.9	*PGI*	TBE
Phosphoglucomutase	2.7.5.1	PGM	TBE
Sorbitol dehydrogenase	1.1.1.14	SODH	CA-8

* E.C. = Enzyme Commission.

† CA-8, gel buffer = 0.074 M Tris, 0.009 M citric acid, pH 8.45 (undiluted); electrode buffer = 1.37 M Tris, 0.314 M citric acid, pH 8.1 (diluted 1:3 for the cathode and 1:4 for the anode); C, gel buffer = 0.002 M citric acid, pH 6.0 (undiluted); electrode buffer = 0.04 M citric acid, pH 6.1 (undiluted) (pH for buffer C is adjusted with N-(3-aminopropyl)-morpholine); CA-7, gel buffer = 0.009 M Tris, 0.003 M citric acid, pH 7.1 (undiluted); electrode buffer = 0.135 M Tris, 0.04 M citric acid, pH 7.0 (undiluted); CA-5.5, gel buffer = 0.064 M Tris, 0.026 M citric acid, pH 5.8 (diluted 1:2); electrode buffer = 0.223 M Tris, 0.093 M citric acid, pH 5.2 (diluted 3:1); TBE = 0.1 M Tris, 0.08 M boric acid, 0.002 M EDTA, pH 8.6 (undiluted) (gel and electrode buffer are identical).

groups.[11] The purpose of the work we report here was to estimate the level of genetic divergence among laboratory populations of *Lu. longipalpis* that originated from three widely separated localities and to determine if post-RIM exist that may be involved in the maintenance of diversity in nature.

MATERIALS AND METHODS

Sand fly colonies

The Brazil strain of *Lu. longipalpis* originated in Lapinha caves near Belo Horizonte, Minas Gerais (approximate coordinates 20°0'S, 44°0'E). The Colombia colony was derived from flies collected by R. B. Tesh near Melgar, Tolima department (4°11'N, 74°18'W) in 1989. The Costa Rica colony was started with flies collected by us near Liberia, Guanacaste province (10°37'N, 85°26'W) in 1991. The locations of collection sites are shown in Figure 1. All colonies were maintained as described by Modi and Tesh.[12] The Colombia and Costa Rica colonies were started with the progeny of more than 100 field collected female flies.

Isozyme analysis

The whole bodies of individual adult flies were homogenized in 7 μl of distilled water. Homogenates were applied to horizontal 12.5% (w/v) starch gels for electrophoresis using standard procedures.[13,14] Five different buffer systems were

FIGURE 1. Localities of the populations that provided sand flies for colonies.

used to maximize electrophoretic separation of enzymes. These are given, along with references, in Table 1. Enzyme-specific histochemical staining procedures were used to visualize bands on gels.[15, 16] Discrete zones of staining activity on gels were assumed to be controlled by single loci coding for specific enzyme products. The products of 27 putative enzyme loci provided adequate resolution to study variability. Loci coding for the same enzyme were designated numerically in sequence from most cathodal to most anodal. Alleles were scored on the basis of the distance bands migrated through the gel with reference to the most common allele, which was given the *rf* value of 1.00.

Hybridization experiments

Virgin females (6 hr or less post pupal emergence) of one colony were placed in a cage with males from another colony. Two days later, flies were fed blood and four days thereafter were transferred to an oviposition container. All intercolony crosses and reciprocals were achieved. Crosses between individuals from the same colony were likewise conducted as controls. Eggs were counted, the larval hatch was estimated, and the mean number of F_1 adults per female was calculated. Sterility in hybrid males was determined by microscopic examination of testes.

TABLE 2

Genetic variability at 27 enzyme loci in three laboratory colonies of Lutzomyia longipalpis

Colony	Mean ± SEM no. of alleles per locus	Mean ± SEM heterozygosity per locus	Proportion of loci polymorphic*
Brazil	1.22 ± 0.08	0.057 ± 0.028	0.222
Colombia	1.33 ± 0.11	0.071 ± 0.027	0.296
Costa Rica	1.44 ± 0.13	0.058 ± 0.026	0.370
Overall	2.00 ± 0.21	0.057 ± 0.018	0.593

* No criterion

TABLE 3

Frequencies at gene loci differentiating colonies of Lutzomyia longipalpis

Locus (*rf*)*	Brazil	Colombia	Costa Rica
ALD			
N	10	10	10
100	1.000	0.000	1.000
81	0.000	1.000	0.000
GOT-1			
N	66	68	76
100	0.007	1.000	0.993
131	0.993	0.000	0.000
28	0.000	0.000	0.007
ME-1			
N	34	34	30
100	0.000	1.000	1.000
92	1.000	0.000	0.000
MPI			
N	19	20	19
100	0.000	0.575	0.447
45	0.000	0.000	0.316
80	0.000	0.425	0.026
120	0.026	0.000	0.211
145	0.974	0.000	0.000
PEP-1			
N	11	15	14
100	0.000	1.000	0.679
109	1.000	0.000	0.321
PEP-2			
N	14	16	16
100	0.250	0.969	0.969
133	0.750	0.031	0.031
PGM			
N	21	24	20
100	0.952	0.083	0.000
125	0.048	0.917	0.000
142	0.000	0.000	0.875
167	0.000	0.000	0.125
SODH			
N	22	15	20
100	0.000	0.167	1.000
67	1.000	0.000	0.000
125	0.000	0.833	0.000

* *rf* = ratio of the distance a band migrates through the gel on mm to the distance that the most common band migrated = 1.00. ALD = aldolase, GOT-1 = glutamate oxaloacetate transaminase-1, ME-1 = malic enzyme-1, MPI = mannose-6-phosphate isomerase, PEP-1 = peptidase-1, PEP-2 = peptidase-2, PGM = phosphoglucomutase, SODH = sorbitol dehydrogenase

The testes and distal portion of the vasa deferentia were dissected and transferred to a small drop of saline on a microscope slide and a cover slip was added. The gross appearance of the testes was noted. Gentle pressure was applied to allow examination of the contents of testes. The preparation was examined under differential interference contrast illumination. Sperm viability was determined based on total numbers and uniformity of morphology as compared with parental males.

RESULTS

Isozyme survey

Eleven of the 27 loci (40.7%) studied were monomorphic throughout the sample. Three of the 16 polymorphic loci were diallelic, and mannose-6-phosphate isomerase was the most polymorphic locus, being coded for by a total of five alleles. The observed mean ± SEM heterozygosity per individual (H) throughout the sample

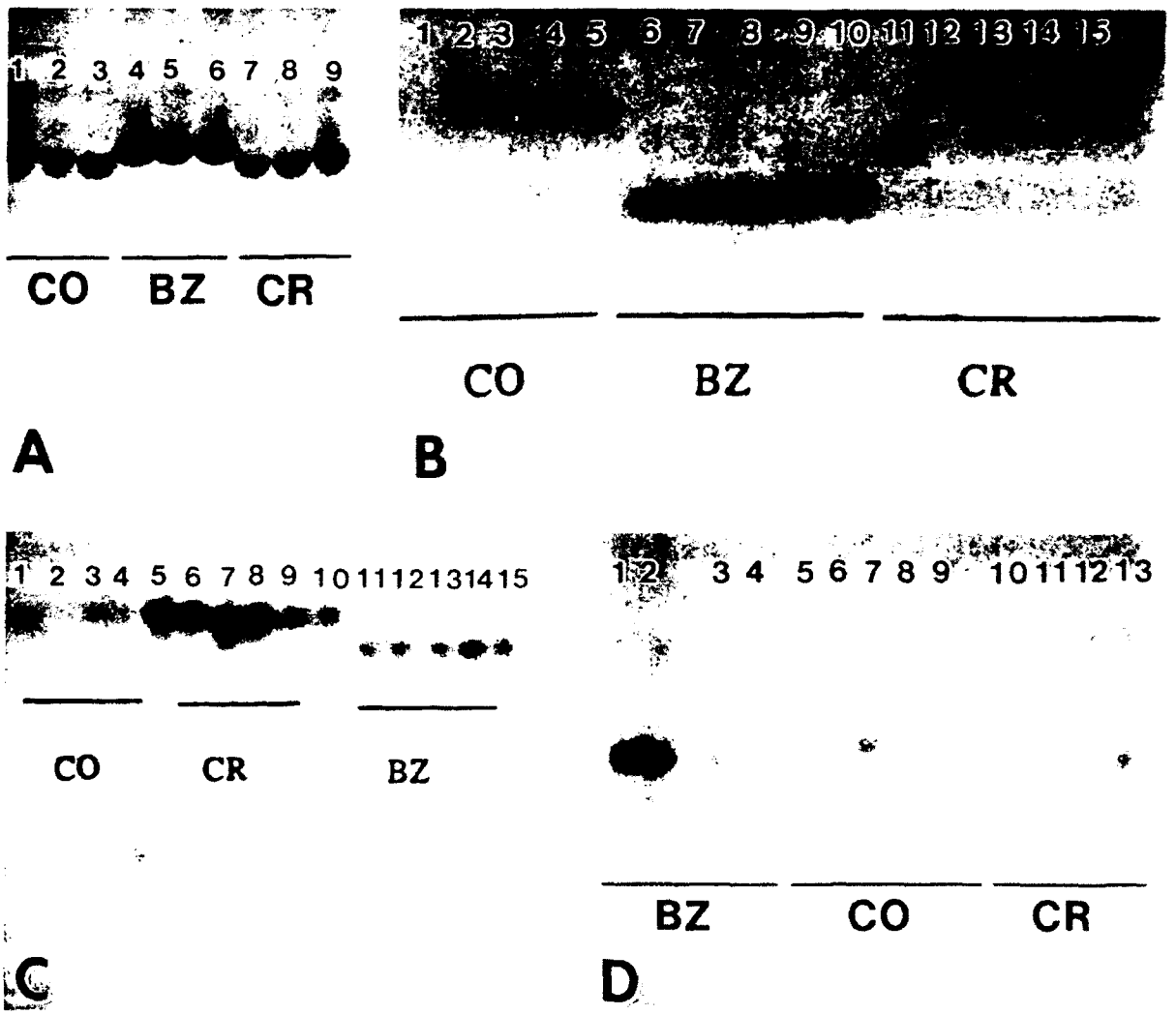

FIGURE 2. Gels illustrating the phenotypes of certain diagnostic loci for species in the *Lutzomyia longipalpis* complex. **A**, malic enzyme (ME). Lanes 1–3 and 7–9 are ME^{100} homozygotes and lanes 4–6 are $ME^{?}$ homozygotes. **B**, mannose phosphate isomerase (MPI). Lanes 1–5 are MPI^{80} homozygotes, lanes 6–8 are MPI^{145} homozygotes, lanes 9 and 10 are MPI^{120}/MPI^{145} heterozygotes, lane 11 is an MPI^{100} homozygote, lane 12 is an $MPI^{?}$ homozygote, lane 13 is an MPI^{80} homozygote, lane 14 is an MPI^{100} homozygote, and lane 15 is an MPI^{80} homozygote. **C**, glutamate oxaloacetate transaminase-1 (GOT-1). Lanes 1–10 are $GOT1^{100}$ homozygotes and lanes 11–15 are $GOT\text{-}1^{133}$ homozygotes. **D**, peptidase-1 (PEP-1). Lanes 1–4 are $PEP\text{-}1^{109}$ homozygotes, lanes 5–9 are $PEP\text{-}1^{100}$ homozygotes, lanes 10–12 are $PEP\text{-}1^{?}$ homozygotes, and lane 13 is a $PEP\text{-}1^{100}/PEP\text{-}1^{109}$ heterozygote (the products of the peptidase-2 locus can be seen as faint bands towards the top of the gel). CO = Colombia; BZ = Brazil; CR = Costa Rica.

TABLE 4

Nei unbiased genetic distances (above diagonal) and genetic similarities (below diagonal), among three Lutzomyia longipalpis *laboratory colonies*

Colony	Brazil	Colombia	Costa Rica
Brazil	–	0.333	0.255
Colombia	0.717	–	0.121
Costa Rica	0.775	0.886	–

was 0.057 ± 0.018 (Table 2). Each of the 16 polymorphic loci carried alleles that were unique to a single colony. Seven loci possessed unique alleles which were fixed, or nearly so, in one colony (Table 3 and Figure 2). Consequently, the levels of genetic divergence among the three colonies, as measured by genetic distance $(\bar{D})$[19], were very high (Table 4). The diagnostic values of loci were calculated using the method of Ayala and Powell;[20] a locus was considered diagnostic if it could be used to correctly assign a fly to the population from which it came with a probability greater than 99%. Using this criterion, there were six loci that were diagnostic for the Brazil and Colombia colonies, five loci diagnostic for the Brazil and Costa Rica colonies, and two loci diagnostic for the Colombia and Costa Rica colonies (Table 5). Using multilocus genotypes, correct identifications can be achieved with a probability exceeding 99.99% for any combination.

Hybridization experiments

Table 6 summarizes the results of hybridization experiments. Among the control crosses, each female laid an average of 21.65 eggs (not counted for the Colombia colony). The mean number of eggs laid among females outcrossed to males of different strains was 29.76. Survival of F_1 generations to the adult stage was lowest in the Brazil colony (22.6%) and was 44.0% in the Costa Rica colony. The mean percent survival to the adult stage among the progeny of five intercolony crosses was 57.7%, which was substantially higher than that observed in the control crosses. In outcrossing colony strains, one might expect increased vigor in the F_1 resulting from heterosis. The reproductive systems of progeny males from control crosses appeared normal and contained numerous mature spermatozoa (Figure 3A). The gross appearance of testes and vasa deferentia in intercolony hybrids did not appear different from those of the progeny of control matings. However, their testes contained only few normal sperm and numerous abnormal ones (Figure 3B). Only in the cross between Colombia females and Costa Rica males were some hybrids observed that had testes containing normal spermatozoa. A sample of F_1 males from this cross were electrophoresed and the gels were stained for enzyme systems that were diagnostic for the Colombia and Costa Rica colonies. The results confirmed that a percentage of these males were in fact derived from Colombia females that were not virgin when used in the cross. Those males that had normal sperm were probably the result of this contamination.

DISCUSSION

We initially analyzed our data by pooling all individual genotypes and treating them as a single population. The level of genetic polymorphism in the overall sample studied was high. Fifty-nine percent of the 27 loci studied carried two or more alleles, however, the mean heterozygosity was relatively low (5.7%, Table 2). The reason for this disparity was that alleles were not distributed randomly among the three colony populations. The degree of genetic structuring among colony populations was profound; every one of the 16 polymorphic loci carried at least one allele that occurred in only a single colony. When the data were partitioned to determine genetic variability for each individual colony, the mean heterozygosity averaged for the three col-

TABLE 5

Diagnostic values of seven loci for distinguishing colonies of Lutzomyia longipalpis*

Comparison	ALD	GOT-1	ME-1	MPI	PEP-1	PGM	SODH
Brazil versus Colombia	1.000	0.999	1.000	0.999	1.000	0.945†	1.000
Brazil versus Costa Rica	†	0.999	1.000	0.999	†	1.000	1.000
Colombia versus Costa Rica	1.000	†	†	†	†	1.000	0.986†

* ALD = aldolase; GOT-1 = glutamate oxaloacetate transaminase-1; ME-1 = malic enzyme-1; MPI = mannose-6-phosphate isomerase; PEP-1 = peptidase-1; PGM = phosphoglucomutase; SODH = sorbitol dehydrogenase.
† Not diagnostic.

TABLE 6

Results of laboratory hybridization between pairs of three laboratory colonies of Lutzomyia longipalpis*

Female × male	Total no. bloodfed	No. of females oviposited	Mean no. of eggs/female	Mean no. of F adults/female	F sperm viability†
Control					
BZ × BZ	48	35	26.5	6.0	+
CO × CO	NC	NC	NC	NC	+
CR × CR	20	17	16.8	7.4	+
Experimental					
BZ × CO	24	16	45.1	27.9	
CO × BZ	23	16	39.8	24.0	+
BZ × CR	26	20	33.7	24.0	+
CR × BZ	48	41	15.8	6.5	+
CO × CR	NC	20	NC	10.5	+
CR × CO	58	50	14.4	7.8	+

* BZ = Brazil; CO = Colombia; NC = not courted; CR = Costa Rica
† + = viable; − = inviable.

onies was 6.2%, essentially the same as for the overall sample. However, the proportion of polymorphic loci decreased from 0.593 in the overall sample to 0.296 on average in the three colonies.

Since the level of genetic divergence between colonies can be best summarized by calculating pairwise values for genetic distance, we used the procedure of Nei[19] (Table 4). Estimates of genetic divergence were quite high. The levels of genetic distance we observed were in fact equivalent to those reported among closely related species in the genus *Lutzomyia* (these included comparisons between *Lu. townsendi*, *Lu. spinicrassa*, *Lu. youngi*, *Lu. longiflocosa*, *Lu. quasitownsendi*, and *Lu. sauroida*).[21] In the same study Kreutzer and others reported that the genetic distance averaged for comparisons between *Lu. longiflocosa*, *Lu. quasitownsendi*, and *Lu. sauroida* was D = 0.010, leading them to suggest that these taxa may be conspecific. The values we observed were an order of magnitude higher than this, suggesting that the level of genetic divergence between pairs of our three colonies more closely resemble the relationships between species in this genus, rather than local populations within a single species.

The comparisons we describe here are among laboratory populations, and it is well known that laboratory colonies are affected by forces that may have a significant impact on their genetic makeup. How much of the genetic divergence we observed may be an artifact of the colonization process? The most common change in colony genetic makeup is decreased heterogeneity.[22] This may result from genetic drift and/or selection. If we consider two colonies of the same species, loss of heterogeneity will significantly impact genetic distance only if an allele at a polymorphic locus approaches fixation in one colony and an alternate allele approaches fixation in the other. In our study, this would have had to have happened at eight different loci (Table 3). This is highly unlikely, considering that two of the colonies were newly established using eggs pooled from more than 100 wild-caught females. The only information available on genetic heterogeneity in natural populations of *Lu. longipalpis* comes from a single population in Bolivia, where observed heterozygosity was measured at 0.037;[23] this value was lower than that for any of our colony populations (Table 2). Thus, if this value is typical, heterozygosity was not likely to have decreased significantly during colonization.

Another phenomenon that may affect the genetic makeup of colony populations is the existence of balanced polymorphisms, which result in excessively high heterozygosity at those loci affected.[22] Excessive heterozygosity at a few loci may obscure otherwise lower than normal values when averaged over all loci. Balanced polymorphisms are the result of linkage to recessive lethal genes, in which case only heterozygotes survive.

An analysis of goodness of fit to Hardy-Weinberg genotypic frequencies was performed for the three colonies. No significant excess of heterozygotes was observed. We believe that the high level of divergence between colonies cannot be adequately explained by independent colonization. We therefore conclude that this divergence reflects the situation in nature, and that our results provide potential genetic markers that should be useful for identification in the field.

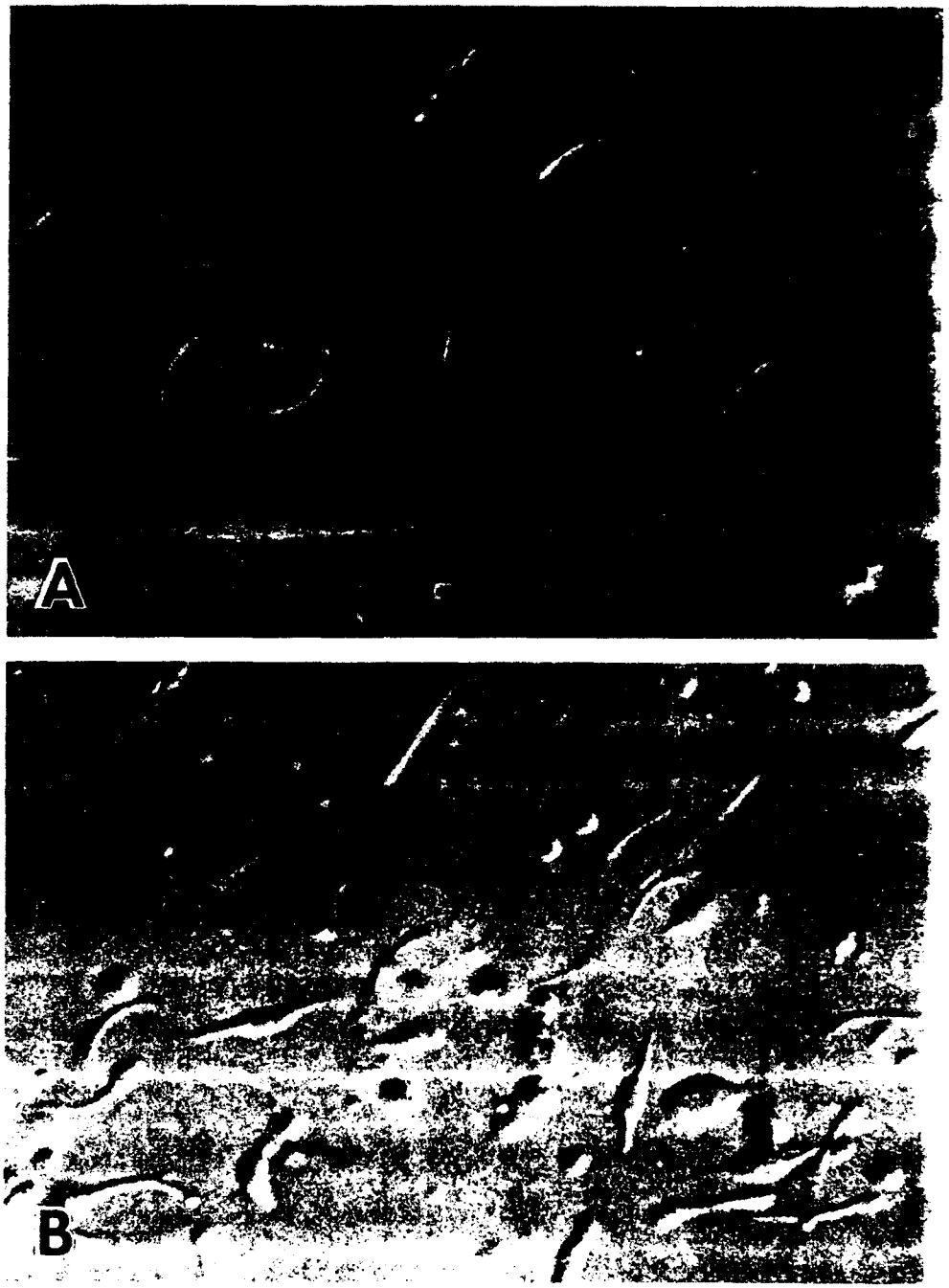

FIGURE 3. A, appearance of normal, mature sperm of *Lutzomyia longipalpis*. B, the typical appearance of the abnormal sperm produced by intercolony F, hybrid males, in this case the product of a cross between a Brazil female and a Colombia male. (Magnification ~ 2,000.)

even in situations where the three species occur in sympatry. It follows that some mechanism must exist that permits high levels of genetic divergence to persist among natural populations. Given the extent of the observed genetic divergence, it is likely that this mechanism severely limits the amount of gene flow among different populations. In addition to geographic isolation, post-RIM would produce such an effect and are amenable to analysis in laboratory situations.

In hybridization experiments, which included all pairwise matings and reciprocal crosses, we observed sterility in male progeny from all intercolony crosses (Figure 3B). In each of the control (intracolony) crosses, males were sexually fertile (Figure 3A). The status of hybrid females

was not assessed. In only one case did some hybrid males appear to be fertile, but this was probably due to experimental error, resulting from failure to separate the sexes from the stock colony before they began mating among themselves. This was evidenced by the presence of homozygotes for maternal allozymes in some F_1 progeny. It is not likely that this mating incompatibility arose in colony because the Colombia colony was established less than two years prior to the time these experiments were conducted and the Costa Rica colony was in the second to sixth generation. Hybrid sterility is a common sequela of interspecific hybridization and is generally considered as an indicator of species status for the two parental forms producing sterile progeny.[23] Our results indicate that *Lu. longipalpis* is not a single species, but exists in nature as a complex of at least three sibling species.

Genetic divergence among vector species can include genes that affect vectorial capacity. The best known examples come from closely related anopheline mosquitoes that vary dramatically in their importance as vectors of malaria. While the overwhelming majority of *L. d. chagasi* infections in Latin America result in life-threatening visceral leishmaniasis, our newly characterized *Lu. longipalpis* species from Costa Rica transmit genetically identical *L. d. chagasi* that cause cutaneous lesions and do not visceralize.[6,7] Preliminary results indicate that the saliva of this species does not contain a vasodilatory peptide (Warburg A and others, unpublished data), that was previously identified in the saliva of the Brazilian species.[24] Because salivary gland lysates from *Lu. longipalpis* (Brazil) have been shown to enhance the development of cutaneous leishmaniasis infections,[25] polymorphisms in salivary composition may be an important factor determining the epidemiology and perhaps pathology of the leishmaniases.

Acknowledgments: We thank R. B. Tesh for the Colombia strain of *Lu. longipalpis*, and Rodolfo Pereira for assistance in the field.

Financial support: This research was supported in part by a MacArthur Foundation grant, "Molecular Biology of Vectors of Malaria and Leishmaniasis," and by grant ID-900389 from the UNDP/World Bank/WHO/Special Programme for Research and Training in Tropical Diseases.

Disclaimer: The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para. 4-3) AR 360-5.

Authors' addresses: Gregory C. Lanzaro and Alon Warburg, Laboratory of Malaria Research, Building 4, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892. Katherine Ostrovska and Phillip G. Lawyer, Department of Entomology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC 20307. Marco V. Herrero, Tropical Disease Research Program, School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica.

Reprint requests: Gregory C. Lanzaro, Laboratory of Malaria Research, NIAID, NIH, Building 4, Bethesda, MD 20892.

REFERENCES

1. Ashford RW, Desjeux P, DeRaadt P, 1992. Estimation of population at risk of infection and number of cases of leishmaniasis. *Parasitol Today 8*: 104–105.
2. Grimaldi G, Tesh RB, McMahon-Pratt D, 1989. A review of geographic distribution and epidemiology of leishmaniasis in the New World. *Am J Trop Med Hyg 40*: 687–725.
3. Corredor A, Gallego JF, Tesh RB, Morales A, Ferro C, Young DG, Kreutzer RD, Boshell J, Palau MT, Caceres E, Palaez D, 1989. Epidemiology of visceral leishmaniasis in Colombia. *Am J Trop Med Hyg 40*: 480–486.
4. Lainson R, Shaw JJ, Ryan L, Ribeiro RSM, Silveira FT, 1985. Leishmaniasis in Brazil. XXI. Visceral leishmaniasis in the Amazon Region and further observations on the role of Lutzomyia longipalpis as the vector. *Trans R Soc Trop Med Hyg 79*: 223–226.
5. Zeledon R, 1985. Leishmaniasis in North America, Central America and the Carribean islands. Chang KP, Bray RS, eds. *Leishmaniasis*. Amsterdam: Elsevier Science Publications, 313–351.
6. Zeledon R, Hidalgo H, Viquez A, Urbina A, 1989. Atypical cutaneous leishmaniasis in a semiarid region of north-west Costa Rica. *Trans R Soc Trop Med Hyg 83*: 789.
7. Ponce C, Ponce E, Morrison A, Cruz A, Kreutzer R, McMahon-Pratt D, Neva F, 1991. Leishmania donovani chagasi: new clinical variant of cutaneous leishmaniasis in Honduras. *Lancet 337*: 67–70.
8. Alexander JB, 1987. Dispersal of Phlebotomine sand flies (Diptera: Psychodidae) in a Colombian coffee plantation. *J Med Entomol 24*: 552–558.
9. Killick-Kendrick R, Rioux JA, Bailly M, Guy MW, Wilkes TJ, Guy FM, Davidson I, Knechtli R, Ward D, Guilvard E, Perieres J, Dubois H, 1984. Ecology of leishmaniasis in the south of France. 20. Dispersal of Phlebotomus ariasi Tonnoir, 1921 as a factor in the spread of visceral leishmaniasis in the Cevennes. *Ann Parasitol Hum Comp 59*: 555–572.
10. Ward RD, Ribeiro AL, Ready PD, Murtagh A, 1983. Reproductive isolation between different forms of Lutzomyia longipalpis (Lutz & Neiva), (Diptera:Psychodidae), the vector of Leishman-

ia donovani chagas Cunha & Chagas and its significance to Kala-Azar distribution in South America. *Mem Inst Oswaldo Cruz 78*: 269–280.

11. Bonnefoy S, Tibayrenc M, Le Pont F, Dujardin J-P, Desjeux P, Ayala FJ. 1986. An isozymic study of Lutzomyia longipalpis (Diptera, Psychodidae), the vector of visceral leishmaniasis in the "Yungas" (Bolivia). *Cah ORSTOM Ser Entomol Med Parasitol 24*: 213–217.
12. Modi GB, Tesh RB. 1983. A simple technique for mass rearing Lutzomyia longipalpis and Phlebotomus papatasi. *J Med Entomol 20*: 568–569.
13. Steiner WWM, Joslyn DJ. 1979. Electrophoretic techniques for the genetic study of mosquitoes. *Mosq News 39*: 35–54.
14. Lanzaro GC, Narang SK, Seawright JA. 1990. Speciation in an anopheline mosquito: enzyme polymorphism and the genetic structure of populations. *Ann Entomol Soc Am 83*: 578–585.
15. Harris H, Hopkins DA. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. New York: North-Holland.
16. Richardson BJ, Baverstock PR, Adams M. 1986. *Allozyme Electrophoresis: a Handbook for Animal Systematics and Population Studies*. San Diego: Academic Press.
17. Clayton JW, Tretiak DN. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J Fish Res Board Can 29*: 1169–1172.
18. Selander RK, Smith MH, Yang SY, Johnson WE, Gentry JB. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I Variation in the old-field mouse (Peromyscus polionotus). *Univ Texas Publ 7103*: 49–90.
19. Nei M. 1972. Genetic distance between populations. *Am Naturalist 106*: 283–292.
20. Ayala FJ, Powell JR. 1972. Allozymes as diagnostic characters of sibling species of Drosophila. *Proc Natl Acad Sci USA 69*: 1094–1096.
21. Kreutzer RD, Palau MT, Morales A, Ferro C, Feliciangeli D, Young DG. 1990. Genetic relationships among Phlebotomine sand flies (Diptera:Psychodidae) in the verrucarum species group. *J Med Entomol 27*: 1–8.
22. Munstermann LE. 1994. Unexpected genetic consequences of colonization and inbreeding: allozyme tracking in Culicidae. *Ann Entomol Soc Am* (in press)
23. Futuyma DJ. 1986. *Evolutionary Biology*. Sunderland, MA: Sinauer Associates, Inc., 111–112.
24. Ribeiro JMC, Vachereau A, Modi GB, Tesh RB. 1989. A novel vasodilatory peptide from the salivary glands of the sandfly Lutzomyia longipalpis. *Science 243*: 212–214.
25. Titus R, Ribeiro JMC. 1988. Salivary gland lysates of the sandfly Lutzomyia longipalpis enhance Leishmania infectivity. *Science 239*: 1306–1308.