Genetic Evidence of a Species Complex in *Anopheles pseudopunctipennis sensu lato*[1]

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This preliminary report provides information concerning genetic variation in the vector mosquito Anopheles pseudopunctipennis sensu lato *that could have malaria control implications. Because of inconsistencies in malaria transmission patterns within geographic zones inhabited by this vector, the possibility that it represents a species complex was investigated. Hybrid crossing studies, electrophoretic analysis of enzyme variation, and DNA restriction studies were carried out in mosquitoes captured in nine areas of Mexico, Bolivia, and Peru. The findings demonstrated the existence of a species complex believed to have resulted from allopatric speciation. This research points to a need for establishing the geographic distribution of the newly recognized species because of their potential influence on malaria control.*

Malaria is on the rise throughout most of the Third World. In Latin America, this trend has been encouraged by conditions resulting from the economic crisis and changes in patterns of development in most countries.

In particular, the public health problem of malaria on the American Continent has worsened since the beginning of the 1980s. The number of cases of malaria reported to the Pan American Health Organization rose from 602 826 in 1980 to a record high of 1 045 808 in 1990 (*1*).

Among the five principal vectors of endemic malaria in the Americas is *Anopheles pseudopunctipennis sensu lato* (2). This mosquito has an extremely broad distribution and occupies a large variety of distinct ecologic niches. In addition, certain inconsistencies stand out in the pattern of malaria transmission within this vector's geographic range (2–7).

Working from the assumption that vector capacity differs in different geographic strains of *An. pseudopunctipennis sensu lato*, research was conducted to assess the possible degree of genetic divergence between different populations of the vector and to determine if distinct genetic forms represented a species complex.

To this end, field collections of *An.*

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pseudopunctipennis sensu lato were carried out in Mexico, Bolivia, and Peru. Specimens were collected during a 13-month period (July 1990–August 1991) from nine clearly distinct geographic areas. Collections were made in mountainous and coastal regions in the states of Morelos, Mexico, and Guerrero in Mexico; in Andean valleys of the provinces (department subdivisions) of Andrés Ibáñez and Arque in Bolivia; and in zones peripheral to urban Lima in Peru.

To evaluate the species complex hypothesis three specific technologies were used: (1) hybrid cross-breeding studies; (2) electrophoretic analysis of enzyme loci; and (3) DNA restriction analysis (RFLP). These procedures were conducted on both field-collected and colony-bred specimens.

Hybridization studies revealed unidirectional hybrid male and female sterility when Mexican females were bred with South American males. Sterility was confirmed by dissection of testes. The testes of F_1 hybrids showed varying grades of abnormality, ranging from the presence of a few partially formed spermatozoa to aspermia (Photos 1A and 1B). The testes were also smaller than normal, and the vas deferens were typically broader and shorter that those of controls (Photos 2A and 2B). Sterility of F_1 female hybrids was inferred by the presence of excessive asynapsis in the polytene chromosomes of the ovarian nurse cells, and also by collapse of the F_2 generation of hybrids, manifested by atrophy of the males' reproductive apparatus.

Electrophoretic analysis provided further evidence of a species complex by showing fixed differences at two enzyme loci, *Gcd* (E.C. 1.1.1.72) and *Pgm* (E.C. 2.7.5.1) out of 16 loci sampled. These differences suggested an absence of gene flow between South American and Mexican populations, the two enzymes distinguishing clearly between individuals from these two populations.

These studies were complemented by genetic distance ($\overline{D}$ or Nei distances) analysis (*8*), which yielded a distance value of 0.13, permitting two clusters to be distinguished. One, from South America, was represented by three populations from Bolivia and Peru; while the other, from Mexico, was represented by six populations (see dendogram and matrix values, Figure 1 and Table 1). The "$\overline{D}$" values are consistent with those found to clearly separate other cryptic anopheline species deemed to show allopatric speciation.

Finally, DNA technology involving the use of ribosomal DNA probes from *Anopheles gambiae* s.s. revealed the presence of a ribosomal DNA fragment in Mexican strains that was absent from South American ones. The heterologous probe was able to differentiate between Mexican male *An. pseudopunctipennis sensu lato* and South American males. The Mexican species of *An. pseudopunctipennis* (species A) was identified by the presence of a 3.4 kilobase fragment after digestion with the restriction enzyme

Figure 1. Dendogram of the presumed relationship among isolated populations of *An. pseudopunctipennis pseudopunctipennis* s.l. from Mexico, Bolivia, and Peru. The dendogram was constructed using the UPGMA method of Sneath and Sokal (*9*).

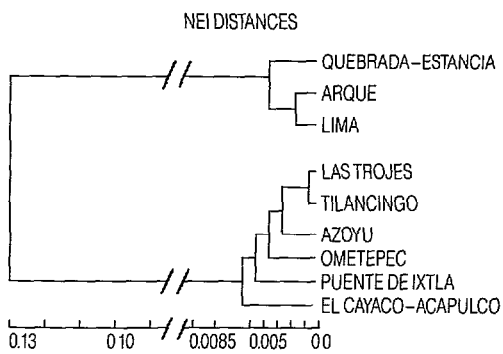

Photo 1A. Normal sperm of F-3 Bolivian colony *An. pseudopunctipennis* mosquito. Note the characteristic long, slender shape and small head. (Picture shown at 650×.)

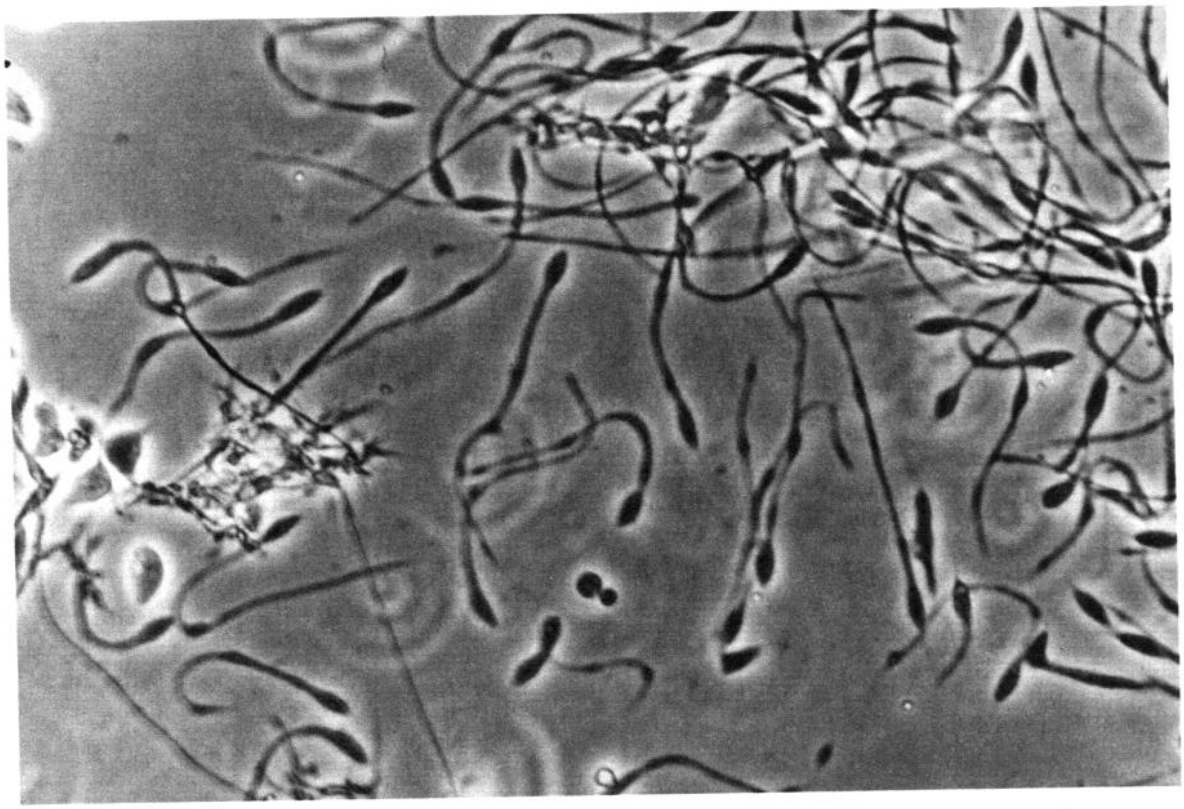

Photo 1B. Abnormal sperm of hybrid from a cross between a Mexican colony female and a Bolivian F-2 colony male. Note the truncated length and large head of most of the sperm. Also note the presence of aberrant forms such as sperm with two heads, sperm with partial development of an extra head on the tail, and spermatocytes with no tails. (Picture shown at 650×.)

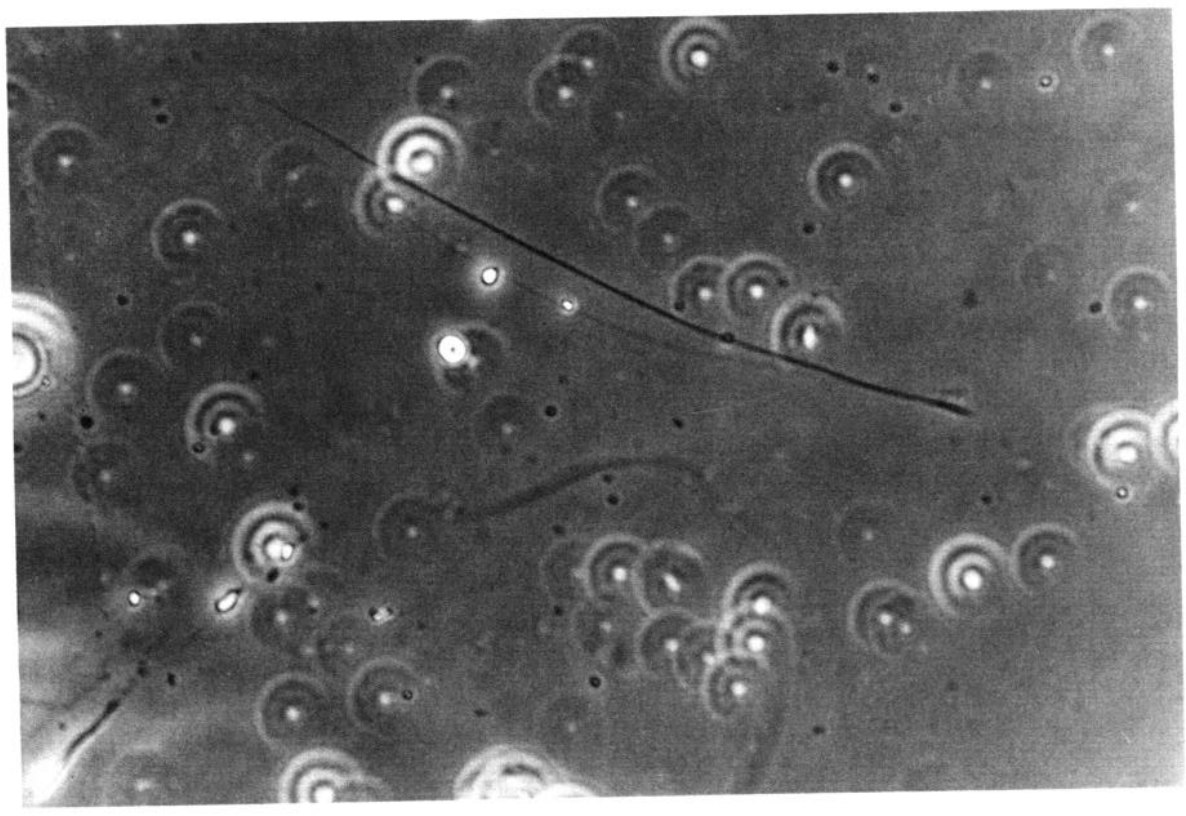

Photo 2A. Normal mosquito testis showing evidence of normal coiled sperm (dark areas). The vas deferens is slender and also clearly full of coiled sperm. (Taken from a male of the Tilacingo, Mexico colony.) (Picture shown at 160×.)

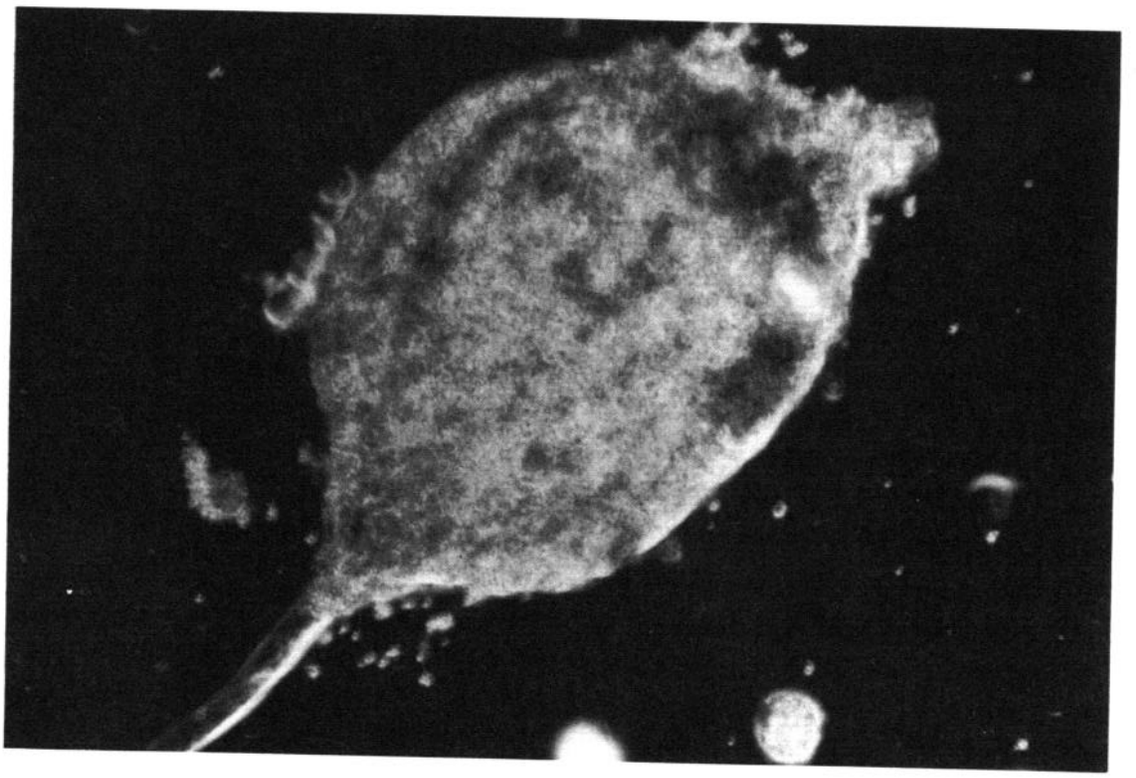

Photo 2B. Atrophied mosquito testis resulting from a cross between a Mexican colony female and a Bolivian wild colony male. Note the broad vas deferens, small size, and absence of sperm contrasting with the testis shown in Photo 2A. (Picture shown at 160×.)

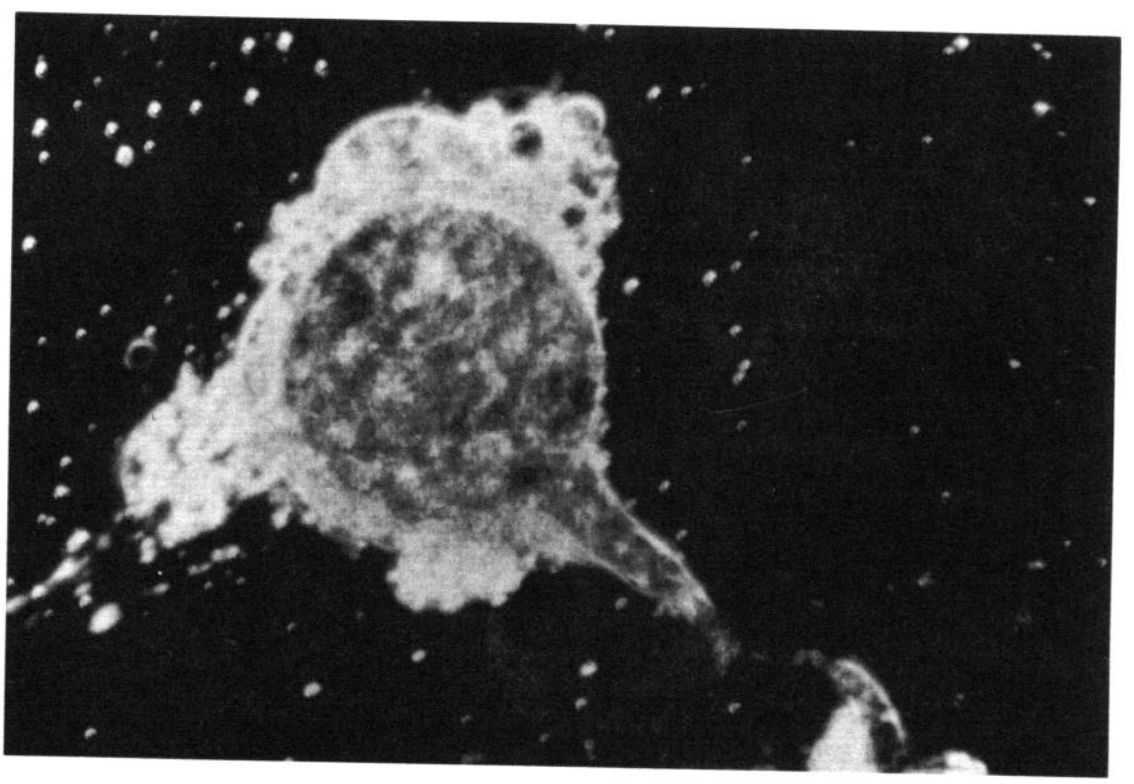

Table 1. Nei's genetic distances and identity matrices among nine populations of *An. pseudopunctipennis* s.l. from Mexico, Bolivia, and Peru.

Country	Locale	1	2	3	4	5	6	7	8	9
Bolivia	1. Quebrada-Estancia	—	.001	.003	.122	.124	.115	.135	.126	.125
Bolivia	2. Arque	.999	—	.001	.129	.132	.123	.143	.133	.133
Peru	3. Lima	.997	.999	—	.132	.136	.126	.146	.136	.136
Mexico	4. Las Trojes	.885	.879	.876	—	.001	.004	.006	.001	.005
Mexico	5. Tilancingo	.883	.876	.873	.999	—	.004	.003	.001	.002
Mexico	6. Puente de Ixtla	.891	.884	.881	.996	.996	—	.010	.005	.004
Mexico	7. El Cayaco	.874	.867	.864	.994	.997	.990	—	.005	.006
Mexico	8. Azoyu	.882	.875	.873	.999	.999	.995	.995	—	.002
Mexico	9. Ometepec	.883	.875	.872	.995	.998	.996	.994	.998	—

EcoRI. This fragment was absent from the South American species.

This body of evidence obtained through a multifaceted approach demonstrates the presence of a species complex in *An. pseudopunctipennis sensu lato*. The two forms identified through these studies have been provisionally named *Anopheles pseudopunctipennis* species A, for the form found on the central plain and Pacific slope of Mexico, and *Anopheles pseudopunctipennis* species B, for the form found among the Andean slopes and intermontane valleys of regions in Peru and Bolivia.

The findings presented here provide the first evidence that *Anopheles pseudopunctipennis sensu lato* constitutes a species complex and suggest allopatric speciation. It is thought that this mechanism of speciation is related to geographic barriers that isolated the populations in Mexico and South America, which in turn could have favored different adaptations by the two populations while preventing gene flow between them (*10*).

These findings provide new information to help resolve pending questions about epidemiologic patterns of malaria transmission in neotropical America. In addition, they have some practical implications that need to be taken into account because of their potential impact on *An. pseudopunctipennis* vector control in the Americas. Specifically, to understand malaria epidemiology and bring about effective vector control, it is necessary to establish the geographic distribution of the two recently recognized species and of any others that future studies may reveal. Such information is critically important for determining the geographic ranges of the different species in the complex, which could affect their relative contributions to malaria outbreaks as well as possible responses to control measures.

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Conference on Children and AIDS

An international conference entitled "Children and AIDS: Medical, Ethical, and Legal Issues" will be held in Onate, Guipúzcoa, in northern Spain, from 13 to 19 June 1993. The conference will be hosted by the International Institute for the Sociology of Law, based in Onate, and is being organized by The American University (Washington, D.C., USA), with the collaboration of the Pan American Health Organization.

The main goal of the meeting is to examine, analyze, and discuss the evolving medical, ethical, legal, and policy issues presented by pediatric AIDS. Particular attention will be given to actual solutions and interventions, so that discussion of pragmatic and applied topics is carefully balanced with presentation of theory, analysis, and pure research. A few of the many issues to be addressed are access to care, the natural history of HIV infection in children, living arrangements for HIV-infected children, peer counseling among adolescents, how to help schools and communities accept and support children with HIV infection, the legal rights of families caring for HIV-infected children, and the funding of prevention, research, and care.

The conference is meant to facilitate the sharing of information and the diffusion of proven or promising approaches among an international audience of practitioners, policy makers, and other professionals. For more information, contact Prof. Emilio Viano, School of Public Affairs/DJLS, The American University, Washington, D.C. 20016-8043, USA; telephone (202) 885-2953; fax (202) 885-1292 or (202) 885-2353; BITNET: EVIANO@AUVM; INTERNET: EVIANO@AMERICAN.EDU.