Two New Subspecies of the *Drosophila willistoni* Group

(Diptera: Drosophilidae)[1]

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The genus *Drosophila* (Drosophilidae, Diptera) is characterized by the existence of groups of two or more sibling species, i.e., species morphologically so similar as to be practically indistinguishable. Dobzhansky (1956) has speculated that the evolution of external morphology has reached in the genus *Drosophila* a high degree of perfection and that the adaptive evolution proceeds largely through physiological channels. This hypothesis has recently been supported by evidence showing that sibling species of *Drosophila* often differ in their allozymes, that is, in enzymes coded for by different alleles at the same genetic locus. Ayala and Powell (1972) have shown that allozymes can be used as species diagnostic characters.

Since little morphological differentiation often exists between closely related species, it is not surprising that the taxonomic category of subspecies has rarely been used in *Drosophila*, in spite of extensive study of the genus. Whenever races or subspecies have been distinguished in *Drosophila*, the distinction has, in most cases, been based on the occurrence of incipient reproductive isolation and/or differences in the frequencies of chromosomal inversions. In this paper I present evidence showing that one pair of subspecies can be distinguished in each of the species, *Drosophila willistoni* and *D. equinoxialis*. The subspecies can be identified by their allozyme patterns, and these are used in their formal description. I believe this is the first time that allozymes have been used in the formal description of a taxon.

REPRODUCTIVE ISOLATION

The *Drosophila willistoni* group contains at least six sibling species endemic in the New World tropics (Spassky *et al.*, 1971). Two of the siblings, *D. insularis* Dobzhansky and *D. pavlovskiana* Dobzhansky and Kastritsis, are narrow endemics; the former on some islands of the Lesser Antilles and the latter in Guyana. The other four species, *D. willistoni* Sturtevant, *D. equinoxialis* Dobzhansky, *D. paulistorum* Dobzhansky and Pavan, and *D. tropicalis* Dobzhansky and Pavan have

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wide geographic distributions, which overlap through Central America and the northern half of continental South America.

The distribution of *D. willistoni* extends from northern Mexico and south Florida, through Central America, the Caribbean islands, and continental South America down to La Plata in Argentina. *D. willistoni* adults were collected in 1954 by Prof. C. Pavan and again in 1972 by Prof. D. Brncic near Lima, Peru. The Lima flies from either sample exhibit incipient reproductive isolation from flies collected in Colombia, Venezuela, Trinidad and Brazil. Crosses having Lima flies as the male parent produce fertile hybrids of both sexes. Females from Lima crossed to males from the other localities yield fertile females but sterile male hybrids. The populations near Lima are therefore assigned to a new subspecies, *D. willistoni quechua* Ayala.

The known distribution of *D. equinoxialis* extends from central Mexico, through Central America, the greater Antilles, and the northern half of continental South America, including the Amazon basin. Flies from Hispaniola, Puerto Rico and Costa Rica, exhibit incipient reproductive isolation from flies collected in eastern Panama or anywhere in continental South America. The populations from Hispaniola, Puerto Rico, and Costa Rica are therefore included in a second new subspecies, *D. equinoxialis caribbensis* Ayala. Crosses between *D. e. equinoxialis* and *D. e. caribbensis* produce fertile females but sterile males.

ENZYME DIFFERENTIATION

Using techniques for starch gel electrophoresis and enzyme assay described elsewhere (Ayala *et al.*, 1972), we have studied genetic variation at 23 to 30 gene loci coding for enzymes. The relevant results are summarized in Table 1. At each of 5 out of 25 loci studied in both subspecies there are substantial differences in the enzyme patterns, and therefore in the genotypic constitution of *D. w. willistoni* and *D. w. quechua*. Also at each of 5 out of 25 loci studied in both subspecies substantial differences exist between *D. e. equinoxialis* and *D. e. caribbensis*. Using the method of Ayala and Powell (1972) it is possible to calculate the probability of correct diagnosis of the subspecies of a single individual of known genotype. This probability ranges from 0.99998 to 0.974 for each of the five diagnostic loci in *D. willistoni*; using jointly the five loci, the probability of incorrect diagnosis of the subspecies of a single individual is 3.4×10^{-14}. The probability of correct attribution for each of the five diagnostic loci ranges in *D. equinoxialis* from 0.9997 to 0.967; using jointly the five loci the prob-

TABLE 1. Allelic frequencies at several diagnostic loci in two pairs of subspecies of *Drosophila*. Alleles designed by their relative electrophoretic mobility; some rare alleles have been omitted from the Table. Sample size is the number of wild genes sampled; i.e., twice the number of wild-collected individuals studied, except for sex-linked loci which exist in males in single dose. The symbols for the loci refer to the enzymes coded for by them, as follows: *Est* = esterase; *Odh* = octanol dehydrogenase; *Xdh* = xanthine dehydrogenase; *Acph* = acid phosphatase; *Mdh* = malate dehydrogenase; *G3pdh* = glyceraldehyde-3-phosphate dehydrogenase; *Hk* = hexokinase.

Subspecies	Sample size	Locus and alleles					Probability of correct diagnosis of the subspecies
A.							
				Est-2			
		98	*100*	*102*	*104*		
D. w. willistoni	7012	.003	.041	.941	.006		.999
D. w. quechua	108	.231	.769	.000	.000		
				Est-4			
		98	*100*	*102*	*104*		
D. w. willistoni	9692	.004	.146	.838	.011		.989
D. w. quechua	114	.000	1.000	.000	.000		
				Est-7			
		96	*98*	*100*	*102*	*105*	
D. w. willistoni	6819	.025	.147	.563	.211	.049	.974
D. w. quechua	59	.847	.000	.153	.000	.000	
				Odh-1			
		96	*100*	*104*			
D. w. willistoni	1088	.039	.882	.071			.994
D. w. quechua	64	.016	.000	.984			
				Xdh			
		95	*97*	*98*	*100*	*101*	
D. w. willistoni	2320	.000	.007	.114	.468	.322	.99998
D. w. quechua	64	.547	.422	.000	.000	.000	
B.							
				Est-4			
		98	*100*	*102*			
D. e. equinoxialis	2682	.150	.769	.081			.967
D. e. caribbensis	2166	.954	.036	.001			

TABLE 1. (*Continued*)

Subspecies	Sample size	Locus and alleles							Probability of correct diagnosis of the subspecies
					Acph-1				
		88	*94*	*100*	*102*	*104*	*106*	*108*	
D. e. equinoxialis	702	.000	.013	.172	.000	.811	.000	.014	.986
D. e. caribbensis	2062	.635	.027	.113	.005	.019	.176	.010	
					Mdh-2				
		94	*106*						
D. e. equinoxialis	1900	.994	.000						.9997
D. e. caribbensis	1572	.0006	.989						
					G3pdh				
		92	*96*	*100*					
D. e. equinoxialis	32	.000	.063	.906					.977
D. e. caribbensis	754	.019	.939	.029					
					Hk-1				
		96	*100*						
D. e. equinoxialis	428	.082	.914						.975
D. e. caribbensis	988	982	009						

ability of misclassification of a single individual of known genotype is 8.0×10^{-11}.

It should be noted that in the formal descriptions which follow, the frequency with which individuals exhibit a given band is calculated on the basis of *genotypic* frequencies. These frequencies can be calculated from the data given in Table 1 by assuming Hardy-Weinberg equilibrium.

Drosophila willistoni Sturtevant

This species becomes the nominate subspecies, *Drosophila willistoni willistoni* Sturtevant.

Drosophila willistoni quechua Ayala, **new subspecies**

Morphologically indistinguishable from *Drosophila willistoni willistoni* as described by Sturtevant (1921), but differing from it by allozyme patterns in electrophoretic assays for following enzymes. (1) *Xanthine dehydrogenase*: in buffer system of pH 9.0, bands migrating anodally less than bands exhibited by *D. w. willistoni*. (2) *Esterase-2*: in buffer system of pH 8.65, bands migrating anodally less than bands exhibited by *D. w. willistoni*. (3) *Octanol dehydro-*

genase-1: in buffer system of pH 9.0, nearly always (more than 99.9 percent of individuals) exhibits band with further anodal migration than common bands of *D. w. willistoni*. (4) *Esterase-4*: in buffer system of pH 8.65, band migrating anodally less than most common band exhibited by *D. w. willistoni*. (5) *Esterase-7*: in buffer system of pH 8.65 nearly always (98 percent of the individuals) with a band migrating anodally less than common bands of *D. w. willistoni*.

Holotype male, laboratory reared, original stock from PERU: Department of Lima, near Lima, collected by net sweeping over fruit baits in June 1972 by Prof. Danko Brncic, reared at Department of Genetics, University of California, Davis by F. J. Ayala, killed 6-IV-1973. Allotype and 497 paratypes (207 males, 290 females), same data as holotype, with paratypes also dated 10-IV-1973, either point mounted or preserved in alcohol. Holotype and allotype, point mounted, deposited in the California Academy of Sciences, Department of Entomology, Type Number 11786. Paratypes deposited in the collections of the California Academy of Sciences, National Museum of Natural History, Washington, D.C., and University of California collections at Berkeley, Davis, and Riverside.

The original stock consisted of 38 inseminated females, maintained in separate cultures. Crosses made among the 38 cultures. The holotype, allotype and paratypes are progenies from these crosses.

Drosophila equinoxialis Dobzhansky

This species becomes the nominate subspecies, *Drosophila equinoxialis equinoxialis* Dobzhansky.

Drosophila equinoxialis caribbensis Ayala, **new subspecies**

Morphologically indistinguishable from *Drosophila equinoxialis equinoxialis* as described by Dobzhansky (1946), but differing from it by allozyme patterns in electrophoretic assays for following enzymes. (1) *Malate dehydrogenase-2*: in buffer system of pH 9.0, nearly always (more than 99.999 percent of individuals) exhibiting band with further anodal migration than most common band of *D. e. equinoxialis*. (2) *Acidphosphate-1*: in buffer system of pH 8.65, nearly always (more than 99.9 percent of individuals) with bands migrating anodally either less (most often) or more than most common band of *D. e. equinoxialis*. (3) *Glyceraldehyde-3-phosphate dehydrogenase*: in buffer system of pH 7.0, nearly always (more than 99.9 percent of individuals) exhibiting bands with less anodal migration than most common band of *D. e. equinoxialis*. (4) *Hexokinase-1*: in buffer system of pH 7.0, nearly always (more than 99.9 percent of individuals) exhibiting bands with less anodal migration than most common band of *D. e. equinoxialis*. (5) *Esterase-4*: in buffer system of pH 8.65, nearly always (more than 99.8 percent of individuals) exhibiting bands with less anodal migration than most common band of *D. e. equinoxialis*.

Holotype male, laboratory reared, original stock from PUERTO RICO: Mayagüez, collected 24-II-1972 by net sweeping over fruit baits by F. J. Ayala, reared at Department of Genetics, University of California, Davis by F. J. Ayala, killed 6-IV-1973. Allotype and 214 paratypes (103 males, 111 females) same data as holotype, with some paratypes killed 10-IV-1973, either point mounted or preserved in alcohol. Holotype and allotype, point mounted, deposited in the California Academy of Sciences, Department of Entomology, Type Number 11787. Paratypes deposited in the collections of the California Academy of Sciences, National Museum of Natural History, Washington, D.C., and University of California collections at Berkeley, Davis, and Riverside.

The original stock consisted of 151 females, maintained in separate cultures. Crosses were made among the 151 cultures. The holotype, allotype and paratypes are progenies from these crosses.

Geographic Distribution: Puerto Rico, Hispaniola and Costa Rica (Limón province).

SUMMARY

Two new subspecies of the *Drosophila willistoni* group are described. The subspecies are established on the basis of partial reproductive isolation (hybrid sterility) between allopatric populations. Members of each pair of subspecies differ from each other by the patterns of their enzymes as assayed by gel electrophoresis. Using five different enzymes, the probability of incorrect identification of the subspecies of an individual of known genotype is less than 1×10^{-10}. This is the first time that allozyme patterns have been used in the formal description of a taxon.

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SCIENTIFIC NOTE

Speculation on the distribution of the Southern California species of *Cafius* with a new record from the Salton Sea (Coleoptera: Staphylinidae)—Members of the genus *Cafius* are found throughout the world along the seashore and on the banks of rivers near the sea. Of the seven species of *Cafius* known from southern California, one (*C. canescens* Mäklin) is abundant, three (*C. seminitens* Horn, *C. lithocharinus* LeConte and *C. luteipennis* Horn) are sometimes common and three (*C. sulcicollis* LeConte, *C. opacus* LeConte and *C. decipiens* LeConte) are rare. *Cafius canescens* is known from Alaska to Baja California, *C. seminitens* and *C. luteipennis* from British Columbia to Baja California, *C. lithocharinus* from Washington to Baja California, *C. sulcicollis* from southern California and Baja California and the very rare *C. decipiens* has been recorded only twice from San Diego (LeConte 1863, Smithson. Misc. Coll., 167: 1–92; Casey 1885, Bull. Calif. Acad. Sci., 1. 205–336). *Cafius sulcicollis*, recorded from Magdelena Island, Baja California (Horn 1894, Proc. Calif. Acad. Sci., 4: 302–449), is the only species known to occur in the southern part of the poorly explored peninsula of Baja California. On March 3, 1968, Kenneth W. Cooper collected three specimens of *C. sulcicollis* at Desert Beach, Salton Sea, Riverside County, California. These specimens are in the collection of the University of California at Riverside. To our knowledge, this is the first record of any species of seashore beetle from the shores of the Salton Sea. *Bledius ferratus* LeConte, a coastal species which has been reported from the Salton Sea (Herman 1972, Bull. Amer. Mus. Nat. Hist., 149: 113–254), lives in salt marshes, not on sea shores, and is widespread in the deserts of southern California. It probably inhabited those areas long before the formation of the Salton Sea.

The Salton Sea was formed in 1904, when the Colorado River overflowed its banks. It is located 235 feet below sea level, in the Colorado Desert about eighty miles inland from the Pacific Ocean over a mountain range whose lowest pass is 2600 feet, and about 100 miles north of the Gulf of California. Its present salinity is slightly greater than that of sea water. The shores of the Salton Sea provide a habitat more like that of the large enbayments of southern Baja California than that of the sea beaches of southern California. The climate of Baja California is semi-desert in the northwest, becoming progressively more arid to the south until that of the middle of the peninsula is similar to the climate of the Colorado Desert except for heavy fogs along the Pacific Coast. It seems likely that *Cafius sulcicollis* (along with *C. decipiens* and *C. opacus*) is distributed along the Pacific shores of southern Baja California, and is uncommon in southern California because that region is at the northern extreme of its range.

Species of *Cafius* are known to feed at least in part on larval Diptera in decaying seaweed (James, Moore and Legner 1971, Trans. San Diego Soc. Nat. Hist., 16: 279–289). Some areas of the collection locality at Desert Beach were covered with a layer of decaying tamarisk needles containing dipterous larvae which could have provided suitable food.

How *Cafius sulcicollis* came to the Salton Sea is a matter of conjecture. This species is a strong flyer which sometimes swarms along the beaches (Leech and Moore 1971, Wasman J. Biol., 29: 65–70). Gravid females possibly could have been carried over the mountains during storms, or they might have been trailered to the Salton Sea in boats. It is unlikely that the species was introduced from the Gulf of California as no member of the genus is known from the northern part of the Gulf.—IAN MOORE AND E. F. LEGNER, *Division of Biological Control, University of California, Riverside, 92502.*

BOOK REVIEWS

THE INSECTS OF AUSTRALIA. Edited by I. M. Mackerras. Melbourne University Press. U. S. Distributor: International Scholarly Book Services, Inc. 1029 p., 704 figs., 8 color plates, Color frontice plate. 1970. $22.50 (U.S.).

The days are past in which a single author can produce a comprehensive textbook in general entomology with an up to date coverage of the higher classification of insects to the family level for any major region. Thus the present book is a product of the efforts of 30 entomologists with diverse specialties. However, this type of approach also has its problems, especially in providing a uniform, coordinated and yet sufficiently concise treatment to fit into a single volume. The efforts of the editor, I. M. Mackerras, and of D. F. Waterhouse, Chief, Division of Entomology, CSIRO who coordinated many of the activities involved, have been highly successful in providing us with a well balanced product. Both men and all the contributors are to be congratulated for their efforts.

This tome of over 1,000 pages is extremely impressive not only in its size and weight, but in the nature and quality of its contents. The first nine chapters of about 200 pages provide a general treatment of the morphology, physiology, cytogenetics, developmental biology, natural history, phylogeny, systematics, and zoogeography of insects. The next 28 chapters of about 750 pages present the systematic treatments of each of the classes of hexapods and orders of insects.

The difficulties of one author producing a comprehensive treatment of general entomology are akin to the difficulties of one reviewer trying to evaluate the efforts of 30 authors. So I will concentrate on the broader aspects of the volume and and leave the detailed criticisms of specific chapters to experts in those areas.

The first nine chapters provide an excellent coverage of the basic aspects of insect structure, function and biology. However, those interested in the applied aspects of entomology will undoubtedly be disappointed in the lack of coverage given to agricultural, medical and forest entomology and biological control. One finds only brief references to the importation of *Cactoblastis* for the control of *Opuntia* and the export of *Rodolia* to California for the control of *Icerya*, both