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A New Subspecies of *Drosophila pseudoobscura*

(Diptera: Drosophilidae)[1]

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Drosophila pseudoobscura Frolowa, which was earlier confused with a European species, *Drosophila obscura* Fallén, was distinguished by its chromosomal complement and some morphological traits by Frolowa and Astaurov (1929). It proved to be one of the most common and ecologically versatile species in western United States, western Canada, Mexico, and Guatemala (Dobzhansky and Epling 1944). Throughout its enormous geographical distribution area, it is morphologically uniform (except for variation in body size, which is due mostly to abundance or scarcity of food and to temperature during the larval development). Most populations are chromosomally polymorphic, and populations from remote localities can sometimes be distinguished by the gene arrangements in their chromosomes (Dobzhansky and Epling 1944, Powell, Levene and Dobzhansky 1972). The interpopulational crosses produce fully fertile hybrids, and there is no evidence of preference for mating with partners of the same geographic origin (Anderson and Ehrman 1969). In the Hooker Lecture read before the Linnean Society of London in 1962, *D. pseudoobscura* was given as an example of a unified, genetically undifferentiated species (Dobzhansky 1963).

In 1960–1962, Prof. Alice S. Hunter found *D. pseudoobscura* far from its previously known distribution area, namely in the highlands above Bogota, Colombia, at elevations of 2200–3280m. The Bogota colony seems to be separated from the nearest point of the main distribution area of the species, in Guatemala, by a gap of some 2400Km. A study of strains from Bogota showed that in chromosomal polymorphism they resembled most the populations from Guatemala, but lacked some of the chromosomal variants that are not uncommon in Guatemala. The Bogota population is also remarkable by having the lightest genetic load of any population of the species (Dobzhansky *et al.*, 1963). Prakash (1972) discovered that male hybrids in the F_1 of the cross

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Bogota ♀ × USA ♂ are completely sterile, while the reciprocal cross gives full fertility; hybrid females are all fertile. He also found no ethological isolation between Bogota and USA flies, and concluded that an incipient reproductive isolation, namely unilateral sterility of hybrid males, has arisen despite "the absence of apparent genic differentiation." Dobzhansky (1974) confirmed the findings of Prakash as far as the F_1 male sterility is concerned, but found a more complex situation in the backcrosses where different chromosomes were marked by suitable mutant genes. One or more genes determining sterility vs. fertility are present in both limbs of the X-chromosome, in the second, and in the third chromosomes. Moreover, while in the F_1 hybrids sterility or fertility are all-or-none phenomena (the sons of Bogota mothers and USA fathers are always sterile, those of USA mothers and Bogota fathers always fertile), in the backcross males the sterility is a threshold character. Some males with identical chromosomal constitution are fertile and other sterile. It remains to be discovered whether this is caused by environmental variations, or by gene modifiers present in the strains used, or by a combination of both. Prakash and Dobzhansky both found that Bogota and USA strains mate at random, at least when tested in experiments in laboratory environments.

It is unprofitable to discuss whether the genetic differentiation between the Bogota and USA races of *D. pseudoobscura* is large or small, unless it can be at least approximately measured. Fortunately, the techniques of gel electrophoresis provide a tool for such analysis. Allelic variation can readily be identified in genes coding for enzymes and other soluble proteins. In recent years, these techniques have become widely used in the fields of population and evolutionary genetics. Gel electrophoresis can also be a powerful tool in systematics. Ayala and Powell (1971) pointed out that allozymes (i.e., enzyme or protein variants coded for by different alleles of the same gene) are good diagnostic characters of sibling species of *Drosophila*, which can hardly or not at all be morphologically distinguished.

Ayala (1973) discovered that allozymes are also good diagnostic characters of *Drosophila* subspecies. In the genus *Drosophila*, subspecies rarely are morphologically distinguishable, although they may exhibit incipient reproductive isolation, generally in the form of partial hybrid sterility. Allozymes were in fact the diagnostic characters used in the formal description of two new subspecies, *Drosophila willistoni quechua* and *D. equinoxialis caribbensis* (Ayala, 1973). In the present note, we use allozymes—as well as certain karyotypic differences—to describe a new subspecies, *Drosophila pseudoobscura bogotana*.

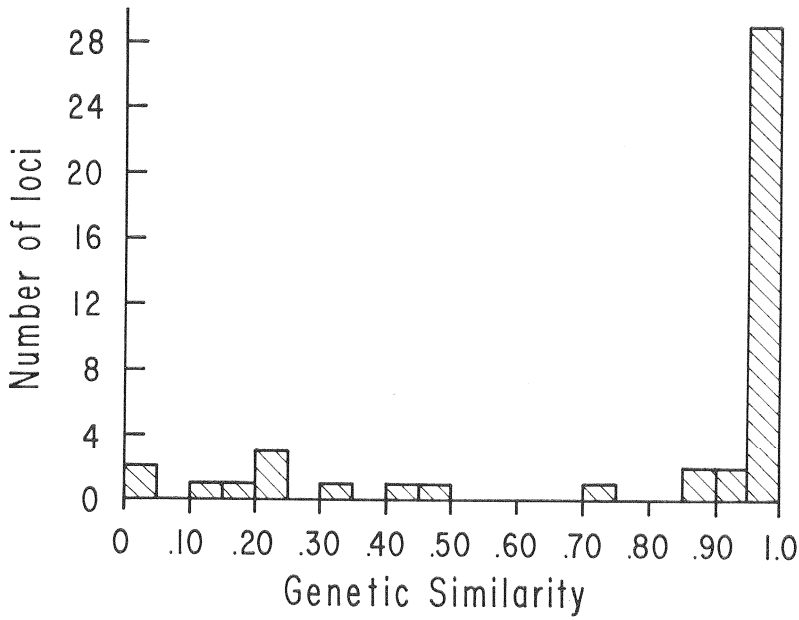

FIG. 1. Frequency distribution of 44 loci relative to genetic similarity (I, after Nei, 1972) for comparisons between the subspecies *Drosophila pseudoobscura pseudoobscura* and *D. p. bogotana*.

ENZYME DIFFERENTIATION

Using standard techniques for gel electrophoresis and enzyme assay (Hubby and Lewontin, 1966; Ayala *et al.*, 1972) 44 gene loci coding for enzymes and soluble proteins have been studied in natural populations of *Drosophila pseudoobscura*. Twenty five loci were studied in our laboratory; data for the other 19 loci are taken from Prakash *et al.* (1969). Five natural populations of *D. p. pseudoobscura* from the United States, and the Bogota population of *D. p. bogotana* were sampled. Genetic similarity, I, and genetic differentiation, D, between the subspecies have been calculated using Nei's (1972) method. The mean values are $I = 0.824$, and $D = 0.194$; that is, on the average about 19 electrophoretically detectable allelic substitutions have occurred for every 100 loci in the separate evolutions of the two subspecies. In contrast, little genetic differentiation exists between local populations of *D. p. pseudoobscura*. I = 0.993, D = 0.007. Figure 1 shows the distribution of loci relative to genetic similarity between the two subspecies. At 29 loci (66% of the total), the two subspecies have essentially identical genetic constitutions, I between 0.95 and 1.00. At two loci

(5%), the differentiation between the subspecies is essentially complete, $I < 0.05$. The similarity between the subspecies at the other 13 loci (30%) ranges from 0.10 to 0.95.

The degree of genetic differentiation between the two subspecies of *D. pseudoobscura* is similar to, although somewhat smaller than, that found between other subspecies of *Drosophila*. Between *D. w. willistoni* and *D. w. quechua*, $I = 0.808$, $D = 0.214$; between *D. e. equinoxialis* and *D. e. caribbensis*, $I = 0.782$, $D = 0.246$. (Ayala and Tracey, 1974; Ayala *et al.*, 1974). Laboratory tests give no evidence of sexual isolation between these subspecies. Crosses between *D. w. willistoni* females and *D. w. quechua* males yield fertile females and males; crosses between *D. w. quechua* females and *D. w. willistoni* males produce fertile females but sterile males. Laboratory crosses between *D. e. equinoxialis* and *D. e. caribbensis* yield fertile females but sterile males, independently of the subspecies of the male parent.

Table 1 gives the allelic frequencies of the six loci which are most different in the two subspecies of *D. pseudoobscura*. 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 0.999 (*Acph-1* locus) to 0.978 (*α-Amy-1*) for the six diagnostic loci listed in Table 1. Using jointly the six loci, the probability of incorrect diagnosis of the subspecies of a single individual is negligible, 5×10^{-15}.

KARYOTYPIC DIFFERENTIATION

As pointed out above, the chromosome pool of the Bogota population is a depauperate variant of the Guatemala populations. Only two gene arrangements in the third chromosomes have been found in Bogota: the TL and SC gene sequences. These are common also in Guatemala and in Mexico (Michoacan), but in Guatemala CU and OA gene arrangements are also found (Dobzhansky and Epling 1944).

It has been known for a long time (Dobzhansky 1937) that at least four cytologically distinguishable kinds of Y-chromosomes are found in *D. pseudoobscura*, and that each of them has a geographic distribution different from the others. The Y-chromosomes of Bogota strains resemble those of the Guatemala populations. They clearly differ from the chromosomes of most of the United States populations, particularly those on the Pacific coast (Figure 2).

These cytological findings make it utterly unprobable that the Bogota population arose from recent introduction by man, presumably with

Table 1. Allelic frequencies at six diagnostic loci in two subspecies of *Drosophila pseudoobscura*. Alleles are designated by the relative electrophoretic mobility of the proteins which they code for. The symbols for the loci refer to the proteins coded, as follows: *Acph-1* = acid phosphatase; *Hk-2* = hexokinase; *Pt-10* and *Pt-8* = larval proteins; *Est-6* = esterase; *α-Amy-1* = alpha amylase. Data for *Pt-10*, *Pt-8* and *α-Amy-1* after Prakash *et al.* (1969).

Subspecies	Locus and alleles						Probability of correct diagnosis of the subspecies
	Acph-1						
	95	*98*	*100*	*104*	*106*		
D. p. pseudoobscura	.038	.000	.947	.000	.014		> .999
D. p. bogotana	.000	.056	.000	.111	.833		
	Hk-2						
	92	*94*	*96*	*98*	*100*	*102*	
D. p. pseudoobscura	.000	.000	.033	.007	.957	.003	> .999
D. p. bogotana	.111	.667	.222	.000	.000	.000	
	Pt-10						
	102	*104*	*106*				
D. p. pseudoobscura	.012	.840	.147				> .989
D. p. bogotana	.000	.000	1.000				
	Pt-8						
	80	*81*	*83*	*85*			
D. p. pseudoobscura	.011	.441	.534	.013			> .981
D. p. bogotana	.870	.100	.030	.000			
	Est-6						
	100	*102*					
D. p. pseudoobscura	.800	.200					> .980
D. p. bogotana	.000	1.000					
	α-Amy-1						
	74	*84*	*100*				
D. p. pseudoobscura	.010	.208	.782				> .978
D. p. bogotana	.000	1.000	.000				

fruits or vegetables from the United States. Far more likely, it came by passive transport, winds or hurricanes, perhaps many thousands or even millions of years ago, from the geographically nearest source, which is Guatemala (Dobzhansky, 1973).

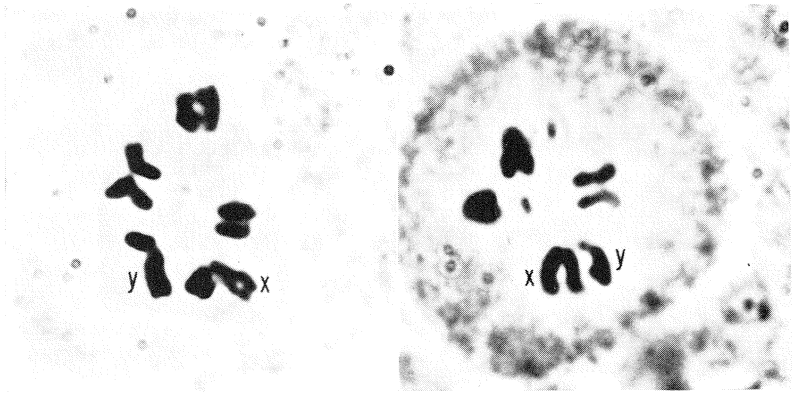

FIG. 2. Spermatogonial metaphase chromosomes of *Drosophila pseudoobscura bogotana* (left) and *Drosophila pseudoobscura pseudoobscura* (right).

DROSOPHILA PSEUDOOBSCURA FROLOWA

This species becomes the nominate subspecies, *Drosophila pseudoobscura pseudoobscura* Frolowa.

Drosophila pseudoobscura bogotana, new subspecies.

Morphologically indistinguishable from *Drosophila pseudoobscura pseudoobscura* as described by Frolowa (1929), but differing from it by enzyme and protein patterns in electrophoretic assays for following.

(1) *Acid Phosphatase-1:* in buffer system of pH 8.65, adult flies exhibit band migrating anodally more than most common bands exhibited by *D. pseudoobscura.* (2) *Hexokinase-2:* in buffer system of pH 7.0, adult flies exhibit bands migrating anodally less than most common band exhibited by *D. p. pseudoobscura.* (3) *Protein-10:* in buffer system of pH 8.9, larvae exhibit band migrating anodally more than most common band exhibited by *D. p. pseudoobscura.* (4) *Protein-8:* in buffer system of pH 8.9, larvae exhibit band migrating anodally less than most common band exhibited by *D. p. pseudoobscura.* (5) *Esterase-6:* in buffer system of pH 8.65, adult flies exhibit band migrating anodally more than most common band exhibited by *D. p. pseudoobscura.* (6) *α-Amylase-1:* in buffer system of pH 8.9, adult flies exhibit band migrating anodally more than most common band exhibited by *D. p. pseudoobscura.* The Y-chromosome is large, submetacentric, apparently like Y's of Guatemala populations, and unlike smaller, submetacentric Y's of most United States populations.

Holotype male, laboratory reared, original stock from COLOMBIA: collected near Bogota by net sweeping over fruit baits in 1968 by Alice S. Hunter, reared at Department of Genetics, University of California, Davis by F. J. Ayala and T. Dobzhansky, killed July 11, 1974. Allotype and 821 paratypes (397 males, 424 females), same data as holotype, with paratypes killed July 8–11, 1974, either point mounted or preserved in alcohol. Holotype and allotype, point mounted,

deposited in the California Academy of Sciences, Department of Entomology, Type Number 12072. Paratypes deposited in the collections of the California Academy of Sciences, National Museum of Natural History, Washington, D.C., and University of California collections Berkeley, Davis, and Riverside.

The original stock consisted of at least six inseminated females whose progenies were maintained in separate cultures. Crosses made among six cultures. The holotype and paratypes are progenies from these crosses.

DISCUSSION

The analogies between *Drosophila willistoni willistoni* and its subspecies *quechua*, and between *Drosophila pseudoobscura pseudoobscura* and its subspecies *bogotana* are striking and worthy of note. The major nominate subspecies are in both cases very common, widely distributed, and only weakly differentiated populations. The minor subspecies *quechua* and *bogotana* are confined to relatively very small areas, and are isolated from the main bodies of their species by geographic barriers. In the case of *quechua* the barriers are the high Andes and the extremely arid coastal zone; *D. willistoni* does not live at high elevations or in parched deserts. The isolation of *bogotana* is due presumably to the intervening tropical lowlands; while *D. pseudoobscura* is abundant on the Pacific Coast of the United States, it withdraws to higher elevations in Mexico and Guatemala.

The hybrid sterility is in both species restricted to only one sex, the male, and to only one of the reciprocal crosses, that in which the minor, narrowly distributed and geographically isolated subspecies, is the female parent. In neither case is there any trace of ethological isolation detectable—the subspecies in the laboratory interbreed freely, despite the production of some sterile hybrids. This is in accord with many previous findings: the premating and the postmating isolating mechanisms are genetically independent. Furthermore, the postmating mechanism (sterility of one sex of hybrids) appears first. So long as the subspecies giving sterile hybrids are securely isolated geographically, there is no need for premating isolation. Females and males of the major and minor subspecies do not meet in nature. Then sterile hybrids are formed solely in laboratory experiments. Only when and if they would expand their areas and come in contact, would there arise a stimulus for natural selection to limit or to interdict their hybridization. Can the subspecies *bogotana* and *quechua* be regarded as incipient species? Only in the sense in which any geographically confined population may conceivably differentiate and diverge from the ancestral stock to become a new species. In this sense any human tribe, especially in preliterate times,

was an incipient species. And yet *Homo sapiens* has preserved its specific unity. Among incipient species there is an enormous "childhood mortality."

SUMMARY

A new subspecies, *Drosophila pseudoobscura bogotana*, is described. This subspecies lives in the highlands above Bogota, Colombia, and is separated by a gap of some 2400Km from the nearest point of distribution of the widely distributed subspecies, *D. p. pseudoobscura*. Male F_1 hybrids between the subspecies are sterile when their mothers are *D. p. bogotana*. The subspecies are genetically differentiated; on the average about 19 allelic substitutions for every 100 loci have occurred in the separate evolution of the subspecies. *D. p. bogotana* also differs from most populations of *D. p. pseudoobscura* in the configuration of the Y-chromosome in spermatogonial metaphase preparations. The subspecies of a single individual of known genotype can be unambiguously identified using the electrophoretic patterns for six gene loci coding for soluble proteins.

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