ORIGIN OF REPRODUCTIVE ISOLATION IN THE ABSENCE OF APPARENT GENIC DIFFERENTIATION IN A GEOGRAPHIC ISOLATE OF *DROSOPHILA PSEUDOOBSCURA*[1]

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ABSTRACT

F_1 males obtained from the cross of *D. pseudoobscura* females from Bogotá (Colombia) × males of this species from mainland, i.e. populations from various locations in the United States and from Guatemala, are sterile. This sterility is due to genes located on the *X* chromosome and the autosomes; the *Y* chromosome is not involved. The percentage of sterile males in backcrosses can be explained by assuming an interaction between two loci on the Bogotá *X* chromosome and probably two loci, one each on two of the mainland autosomes. The role of founder events, inbreeding and geographic isolation in the development of reproductive isolation and the magnitude of gene differences responsible for the origin of reproductive isolation is discussed. It is concluded that founder events, inbreeding and geographic isolation play a major role in the development of reproductive isolation and that major adaptive incorporation of new alleles at a large number of structural loci is not necessary for the origin of reproductive isolation.

ONE of the most significant questions of evolutionary biology is the manner by which species are formed and the kind and magnitude of the genetic difference that characterizes the formation of species. Among evolutionary biologists, it is now widely accepted that animal species are formed by genetic divergence of different geographical populations; the genetic divergence in different populations of the species occurs as an adaptive response to local environmental conditions. In addition, MAYR (1963) has emphasized the role of founder events and geographical isolation in the process of speciation. According to MAYR, different geographical populations with gene flow among them have little if any chance of developing reproductive isolation. It is in a complete isolate started by a few founders that reproductive isolation might arise. The second aspect of our question concerns the nature of genetic differences which are responsible for the development of reproductive isolation in different populations of a species and the cause(s) of the origin of reproductive isolation. According to MAYR (1963), reproductive isolation arises as an incidental by-product of genetic reconstitution in the isolated populations, which occurs in response to the events of founder effect and adaptation to the local environment.

In order to understand the geographic mode of speciation and the nature of

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genetic differences between species, we examined genetic variation at 24 loci by electrophoresis of proteins in central, marginal and isolated populations of *Drosophila pseudoobscura* (PRAKASH, LEWONTIN and HUBBY 1969) and a population of the closely related sibling species *Drosophila persimilis* (PRAKASH 1969). Except for loci associated with the third-chromosome inversion polymorphism (PRAKASH and LEWONTIN 1968, 1971), no genetic differentiation was observed between North American mainland populations from California, Colorado and Texas. On the other hand, the Bogotá (Colombia) population, which is completely isolated from the main body of the species distribution by no less than 1500 miles (DOBZHANSKY *et al.* 1963) shows a drastic reduction in the proportion of polymorphic loci and the amount of heterozygosity. In most cases, the most frequent allele of the mainland populations is fixed in Bogotá and there is no locus in the Bogotá population which has entirely different allele(s) from those found in the mainland; we did not even observe one unique allele at any of the 24 loci. Comparison of 24 homologous loci of *D. pseudoobscura* from the mainland populations and of a population of *D. persimilis* showed that a few unique alleles were present in *D. persimilis* but no locus in *D. persimilis* was entirely unique in its alleles (PRAKASH 1969).

We then checked if different widely separated populations of *D. pseudoobscura* from California, Colorado, Texas, Guatemala and Bogotá show any sign of speciation. We have found that the Bogotá population of *D. pseudoobscura* shows one way sterility of hybrid males; when females of Bogotá are crossed to males from any of the mainland U.S. or Guatemalan populations, we obtain sterile F_1 males. The reciprocal cross of Bogotá males $\times$ mainland females gives fertile F_1 males. The F_1 females from both crosses are fertile. These results are relevant for answering questions about the mode of origin of reproductive isolation (speciation) and the amount and nature of genetic changes involved in this process. From our observations, we conclude that founder effect and complete isolation of the population from the main body of the species are important prerequisites for the process of speciation and that the development of reproductive isolation does not require incorporation of new alleles at a large number of genetic loci.

RESULTS

Mating choice experiments: Mating preferences were examined in mating choice experiments in plexiglass chambers described in PRAKASH (1967). Ten virgin females and ten virgin males, five days old, from each of two strains used in a particular experimental set up, from two different populations, were left together for up to 30 min. The copulating pairs were removed by aspiration and the time and kind of mating was recorded. The wings of flies were marked with holes to assist in identification. Five to ten strains from each population were used in these studies.

Table 1 gives the results. There is no significant deviation from random mating in any of the experiments. Matings between flies from different populations were as frequent as matings between flies from the same population. In extensive

TABLE 1

Mating choice experiments between strains from mainland United States and Bogotá (Colombia) populations of D. pseudoobscura

Cross Strain 1 × Strain 2	Number of chambers run	Number of matings	Numbers of each type of mating 1♀×1♂	1♀×2♂	2♀×1♂	2♀×2♂	χ^2 for random mating (1 d.f.)
Strawberry Canyon, Berkeley × Mesa Verde	5	33	10	5	10	8	0.50
Strawberry Canyon, Berkeley × Austin	8	42	9	10	8	15	0.67
Strawberry Canyon, Berkeley × Bogotá	8	43	15	9	11	8	0.097
Mesa Verde × Austin	13	64	20	16	11	17	1.72
Mesa Verde × Bogotá	15	80	27	19	15	19	1.66
Austin × Bogotá	15	53	18	11	12	12	0.78

experiments on mating preferences of *D. pseudoobscura* from British Columbia, California, Colorado, Mexico and Texas, ANDERSON and EHRMAN (1969) found no excess of homogamic matings.

F_1 male sterility: We then checked if the F_1 offspring obtained from interpopulation crosses are fertile. Ten to 15 pairs of sexually mature adults were allowed to lay eggs and the F_1's were checked for fertility by mass (1) $F_1 \times F_1$ matings and (2) backcrossing separately F_1 males and F_1 females to the parental strains. F_1 males and females obtained from interpopulation crosses between Strawberry Canyon (Berkeley) California; Mesa Verde, Colorado; Austin, Texas; and Guatemala were fertile; F_1 males and females obtained from crossing mainland *D. pseudoobscura* females (Strawberry Canyon, Mesa Verde, Guatemala) to Bogotá males were also fertile (Table 2A). But when the Bogotá females are crossed to mainland males, it was observed that mass matings of $F_1 \times F_1$ thus obtained gave no larvae (Table 2B). When F_1 males and females were separately backcrossed to the parental strains, it was found that the F_1 males were totally sterile, and the F_1 females were fertile. In these experiments, we used 10 different strains of Bogotá and a large number of different strains of the mainland populations. Out of a total of 22 different crosses between mainland and Bogotá strains, F_1 males were found to be sterile in all crosses. The sterility of F_1 males obtained from matings between Bogotá ♀♀ × mainland ♂♂ is not due to their lack of mating ability since a large number of these F_1 males were observed mating. Phase contrast microscopic examination of testes from sterile F_1 males showed that the sperm in the testes were of much smaller size than in the fertile males of corresponding age. Usually the sperm tails were ¼ as long as the sperm tail length in normal males, or even shorter, and usually the sperms were not motile.

Genetic basis of male sterility: Since the reciprocal crosses between Bogotá and mainland populations differ, the F_1 male sterility could be due to either cytoplasmic or chromosomal factors with interaction between the *X* and *Y* chromosomes and/or the autosomes. To understand the genetic basis of hybrid male

TABLE 2

Fertility of F_1's obtained from interpopulation crosses
Figures in parenthesis indicate number of different strains used

Female parents × Male parents		Number of crosses involving different strain combinations	Fertility of $F_1 \times F_1$ matings
A.			
Strawberry Canyon (4) ♀ ♀	× Austin (4) ♂ ♂	4	Fertile
Austin (4) ♀ ♀	× Strawberry Canyon (4) ♂ ♂	4	Fertile
Austin (4) ♀ ♀	× Guatemala (4) ♂ ♂	4	Fertile
Guatemala (4) ♀ ♀	× Austin (4) ♂ ♂	4	Fertile
Strawberry Canyon (4) ♀ ♀	× Guatemala (4) ♂ ♂	4	Fertile
Guatemala (4) ♀ ♀	× Strawberry Canyon (4) ♂ ♂	4	Fertile
Strawberry Canyon (10) ♀ ♀	× Bogotá (10) ♂ ♂	10	Fertile
Mesa Verde (10) ♀ ♀	× Bogotá (10) ♂ ♂	10	Fertile
Guatemala (4) ♀ ♀	× Bogotá (4) ♂ ♂	4	Fertile
B.			
Bogotá (10) ♀ ♀	× Strawberry Canyon (10) ♂ ♂	10	Sterile
Bogotá (8) ♀ ♀	× Mesa Verde (8) ♂ ♂	8	Sterile
Bogotá (4) ♀ ♀	× Guatemala (4) ♂ ♂	4	Sterile

sterility, F_1 hybrid females with Bogotá cytoplasm (obtained from the cross Bogotá ♀ ♀ × mainland ♂ ♂) or with mainland cytoplasm were backcrossed separately to Bogotá males and to mainland males. The backcross males obtained from these crosses were tested for fertility by mating 2–3-day old individual males with 3 virgin females. The flies were changed to fresh vials every third day; such changes were made 4–5 times and the flies were allowed to stay in the last vial from 15–20 days. The vials were examined for the presence of larvae and if no larvae were observed in any of the vials, then the male was classified as sterile. Table 3 gives the results. The percentage of sterile males in BC_1 is the same regardless of the cytoplasm of the F_1 hybrid females. The sterility of males, then, does not involve cytoplasmic factors but is due to chromosomal factors. If the sterility were due to interaction between one locus on the *X* chromosome of Bogotá and a locus (or loci) on the *Y* chromosome of mainland, then we would expect that 50% of the backcross males obtained from the cross F_1 hybrid ♀ ♀ × mainland ♂ ♂ would be sterile. We would also expect no sterility in backcross males obtained from crosses between F_1 hybrid females × Bogotá males. Our results given in Table 3 do not agree with these expectations. We observe that only 30% of the backcross males obtained from backcrossing F_1 hybrid females to mainland males are sterile and 14% of the BC_1 males obtained from backcrossing F_1 hybrid females to Bogotá males are sterile. Since we observe that 14% BC_1 males with the *Y* chromosome of Bogotá are sterile (Table 3B) we may conclude that the *Y* chromosomes plays no role in male sterility; if the *Y* chromosome were involved in hybrid male sterility then we would expect all of BC_1 males with the *Y* chromosome of Bogotá to be fertile since we observe almost no

TABLE 3

Percent fertility of BC_1 males obtained from backcross of F_1 hybrid (Bogotá ♀ ♀ × mainland ♂ ♂ or the reciprocal cross) females

Cross	BC_1 ♂♂ Percent fertile	BC_1 ♂♂ Percent sterile	Total BC_1 ♂♂ tested
A.			
F_1 hybrid ♀ ♀ (Bogotá cytoplasm) × mainland ♂ ♂	67	33	247
F_1 hybrid ♀ ♀ (mainland cytoplasm) × mainland ♂ ♂	71	29	367
Average (1)*	70	30	614
B.			
F_1 hybrid ♀ ♀ (Bogotá cytoplasm) × Bogotá ♂ ♂	86	14	253
F_1 hybrid ♀ ♀ (mainland cytoplasm) × Bogotá ♂ ♂	86	14	149
Average (2)*	86	14	402

* $\chi^2_{(1)}$ between totals of 1 and 2 = 38.35 $P < 0.005$.

sterility in F_1 hybrid males with the *Y* chromosome of Bogotá; 80 such males were tested individually for sterility; only two males were found sterile. BC_1 hybrid females (obtained from backcrossing F_1 hybrid females with either Bogotá or mainland cytoplasm to the males from mainland) were then mated to either mainland or Bogotá males separately. The BC_2 males obtained from such crosses were tested for sterility in the same manner as that used for the BC_1 males. It is observed that the crosses BC_1 hybrid female × mainland male produce 14–16% sterile BC_2 males; 5% of BC_2 males obtained from the cross BC_1 hybrid female × Bogotá male are sterile (Table 4). Since we have already excluded the *Y* chromosome, the possible mechanism of male sterility is an interaction between the *X* chromosome and the autosomes.

Experiments were then done to ascertain the role of autosomes in hybrid male sterility. For this purpose, males which had the *X* chromosome of Bogotá, the *Y* chromosome of mainland and different combinations of mainland and Bogotá autosomes were obtained by the following scheme of crosses: F_1 hybrid females, which were obtained by crossing Bogotá females × Strawberry Canyon, California males, were backcrossed to Strawberry Canyon males. One-hundred-twenty BC_1 males obtained from this cross were then backcrossed to Bogotá females. All BC_2 males thus obtained will have the intact *X* chromosome of Bogotá which has undergone no recombination with the mainland *X* and the *Y* chromosome of

TABLE 4

*Percent fertility of BC_2 males obtained from backcrosses of BC_1 hybrid females**

Cross	BC_2 ♂♂ Percent fertile	BC_2 ♂♂ Percent sterile	Total BC_2 ♂♂ tested
BC_1 hybrid ♀ ♀ (Bogotá cytoplasm) × mainland ♂ ♂	86	14	238
BC_1 hybrid ♀ ♀ (mainland cytoplasm) × mainland ♂ ♂	84	16	375
BC_1 hybrid ♀ ♀ (Bogotá cytoplasm) × Bogotá ♂ ♂	95	5	101

* BC_1 hybrid females were obtained from backcrossing F_1 hybrid females to the mainland males.

mainland, but will differ in their autosomal combinations. If the autosomes did not play any role in male sterility or male fertility, then we would expect all BC_2 males to be sterile. Out of a total of 160 BC_2 males tested for fertility by mating each BC_2 male singly to 3 virgin Strawberry Canyon females, 40% of the BC_2 males were found to be fertile and 60% were sterile. These results show that there are genes on the autosomes which participate in hybrid male fertility. Since the autosomes were not marked in these experiments, we cannot tell which autosome combinations produce fertile males. If the sterility in BC_2 males were due to an interaction of the *X* chromosome with only one particular autosome pair, then we would expect 75% of these males to be sterile, since 75% of these males will be heterozygous for a specific autosome pair. If two of the three major autosomes are involved, such that males heterozygous for both autosomes are sterile, then we would expect 56% of BC_2 males to be sterile; this expectation is fairly close to the observed sterility of 60%. We then assume that at least two of the three major autosomes are involved in hybrid male sterility and mainland autosomes show dominance for sterility when present with the *X* chromosome of Bogotá.

Crosses were then made to check the role of the *X* chromosome in hybrid male sterility. Mainland females heterozygous in the *X* chromosome for the wild-type and sex-ratio gene arrangements were mated to Bogotá males. Recombination is almost completely suppressed in the right arm of the *X* chromosome in females heterozygous in the *X* chromosome for the wild-type and sex-ratio gene arrangements, but is not affected much in the left arm (STURTEVANT and DOBZHANSKY 1936). F_1 "sex ratio" males were backcrossed to Bogotá females. Backcross 1 females obtained from this cross will be heterozygous for the sex-ratio *X* chromosome of mainland and wild *X* chromosome of Bogotá. These BC_1 females were crossed with the mainland males. The BC_2 males will all have the *Y* chromosome of mainland: ignoring viability differences, 50% of these males will have the

X chromosome of mainland and the other 50% the *X* chromosome of Bogotá. In these experiments, in addition to sex-ratio inversions, the *XR* chromosome is also marked with the Esterase alleles. The mainland *XR* chromosome thus bears the sex ratio inversion and Esterase-$5^{1.04}$ allele and the *X* chromosome of Bogotá is marked with the wild-type gene arrangement and Esterase-$5^{1.0}$ allele. The BC_2 males were classified for their *XR* chromosome and fertility. The results are given below.

XR Chromosome	Percent fertile	Percent sterile	Total BC_2 males
Bogotá	24	76	189
Mainland	100	—	130

Of 319 BC_2 males tested, 55% were fertile and 45% were sterile. All sterile males had *XR* chromosome of Bogotá; this was determined from analysis of the Esterase genotype of these males. The *XR* genotypes of fertile males were determined by an examination of sex ratio in the offspring. Since all BC_2 males with the *XR* chromosome of mainland are fertile and 76% of BC_2 males with the *XR* chromosome of Bogotá are sterile, we suppose for the present that genes responsible for hybrid male sterility are located on the *XR* chromosome. If we assume now that male sterility is caused by interaction of genes on the *XR* chromosome and at least two of the major autosomes, then we expect all BC_2 males with the *XR* chromosome of Bogotá to be sterile since all these males carry at least one of each autosome from mainland. But we find that 24% of BC_2 males with the *XR* chromosome of Bogotá are fertile. These fertile BC_2 males probably carry recombinant *X* chromosomes which have the left arm of mainland and right arm of Bogotá. Some of the fertile BC_2 males with *XR* of mainland must then carry *XL* of Bogotá as a result of recombination. It seems then that if only the left or the right arm of the *X* chromosome is from mainland, fertile males will be produced regardless of the autosomal constitution. For the present we then suppose that the *X* chromosome has genes on both arms which play a significant role in hybrid male sterility. However, this view will have to be modified later in this section.

We can explain the results of backcross male sterility satisfactorily if we assume that there are two loci on the *X* chromosome which recombine with a frequency of 0.25 and one locus on each of the two autosomes which interact to produce male sterility. The experiments on the role of *X* chromosome in male sterility show that 24% of the BC_2 males which carry the Bogotá *XR* chromosome are fertile. According to our hypothesis we expect 25% of these BC_2 males to have recombinant *X* chromosomes which have the left arm of mainland and the right arm of Bogotá. Males possessing either the left or the right arm of *X* chromosome from mainland are expected to be fertile. The results of sterility in BC_1 and BC_2 males given in Tables 3 and 4 can be similarly explained. Table 5 gives the observed and expected values of sterility in backcross males. Of the BC_1 males in cross 1, 37.5% are expected to have the parental Bogotá *X* chromosome; such BC_1 males are expected to be sterile since they all have at least one of the autosomes of mainland for each autosome pair. In cross 2, we expect $\frac{1}{4} \times .375 = 9.5\%$ BC_1 males to be sterile, since $\frac{1}{4}$ of the BC_1 males which carry the parental Bogotá *X* chromosome would be heterozygous for two pairs of autosomes. In

TABLE 5

Observed and expected sterility in backcross males in percent
Expected sterility is based on assumption of two loci on the *X* chromosome with $r = 0.25$ and one locus on each of two of the autosomes (see text for further details)

Cross number	Type of males	Observed sterility	Expected sterility
1	BC_1 males obtained from backcross of F_1 hybrid ♀ ♀ to mainland ♂ ♂ (see Table 3)	30	37.5
2	BC_1 males obtained from backcross of F_1 hybrid ♀ ♀ to Bogotá ♂ ♂ (see Table 3)	14	9.5
3	BC_2 males obtained from backcross of BC_1 hybrid ♀ ♀ to mainland ♂ ♂ (see Table 4)	14–16	14.0
4	BC_2 males obtained from backcross of BC_1 hybrid ♀ ♀ to Bogotá ♂ ♂ (see Table 4)	5	7.8

cross 3 we expect $.375 \times .375 = 14\%$ BC_2 males to carry the Bogotá *X* chromosome. All these males would be sterile since they all have at least one of the autosomes of mainland for each autosome pair. Similarly we expect 14% of BC_2 males in cross 4 to carry the Bogotá *X* chromosome. Of these 14% males, only 56% will be heterozygous for two pairs of autosomes. The expected sterility in these BC_2 males then is $.14 \times .56 = 7.8\%$. The observed sterility in BC_1 and BC_2 males is in fair agreement with our hypothesis.

From our observations we can now be more specific about the location of genes on the *X* chromosome; one of the two loci in the *X* chromosome cannot be far into the right arm, because $r = 0.25$ between these two loci can explain results of sterility in backcross males, regardless of whether the right arm in the mothers of these males could recombine freely as in crosses in Tables 3 and 4, or recombination was prevented almost entirely due to heterozygosity of the right arm for wild and sex-ratio gene arrangements. Inversion heterozygosity in *XR* suppresses recombination in the left arm near to the centromere, but recombination is not affected in most of the *XL* chromosome (STURTEVANT and DOBZHANSKY 1936). It means then, that the two loci on the *X* chromosome are most likely to be located on the left arm, one near the centromere and the other about $r = 0.25$ distance towards the tip of the left arm of the *X* chromosome.

In conclusion, our results of percent sterility in backcross hybrid males obtained from several different kinds of crosses are fairly consistent with the hypothesis that there are two genes on the left arm of the *X* chromosome, one located near the centromere and the other about $r = 0.25$ distance away and one locus on each of the two particular autosomes which interact to produce male sterility. Further experiments are needed to check the validity of this hypothesis.

Sex Ratio in F_1 hybrids and backcross progeny: Table 6 gives the results of sex ratio studies. We observe a slight excess of females in F_1 hybrids and in BC_1 progeny. (Table 6A, B, C, D). These BC_1 progeny were obtained by backcrossing F_1 hybrid males to either mainland or Bogotá females. The BC_1 progeny obtained by backcrossing F_1 hybrid females gave only 36% males (Table 6E); the propor-

TABLE 6

Percent sex ratio in hybrid and backcross progeny

Cross	Percent of females	Percent of males	Total number of progeny
A	58	42	1349
B	55	45	410
C	51	49	1842
D	55	45	910
E	64	36	168
F	82	18	4122

A: F_1 progeny from the cross: Bogotá ♀ ♀ × Strawberry Canyon ♂ ♂. B: F_1 progeny from the cross: Strawberry Canyon ♀ ♀ × Bogotá ♂ ♂. C: BC_1 progeny from the cross: F_1 hybrid ♂ ♂ (obtained from the cross: Strawberry Canyon ♀ ♀ × Bogotá ♂ ♂) × Strawberry Canyon ♀ ♀. D: BC_1 progeny from the cross: F_1 hybrid ♂ ♂ obtained as in C × Bogotá ♀ ♀. E: BC_1 progeny from the cross: F_1 hybrid ♀ ♀ (obtained from the cross: Bogotá ♀ ♀ × Strawberry Canyon ♂ ♂) × Strawberry Canyon ♂ ♂. F: BC_3 progeny obtained as follows: BC_1 male progeny of E were crossed to Bogotá ♀ ♀. BC_2 male progeny were then mated individually to Strawberry Canyon females and the sex ratio was studied in BC_3 progeny.

tion of males was further reduced to 18% in BC_3 progeny, (Table 6F). The male inviability observed in BC_1 and BC_3 (Table 6E,F) presumably occurs as a result of unfavorable chromosome interactions. There is, then, considerable hybrid breakdown as evidenced by the decline in viability of backcross males.

DISCUSSION

As has been pointed out earlier mainland U.S. populations of *D. pseudoobscura* are genetically very similar except for the loci which are associated with the third-chromosome gene arrangements (PRAKASH and LEWONTIN 1968, 1971; PRAKASH *et al.* 1969). The allele frequencies and amount of polymorphism are similar in all mainland populations. The Bogotá population, on the other hand, shows a drastic reduction in genetic variation; there is loss of polymorphisms and the average heterozygosity of an individual from Bogotá is only 4.4% as opposed to 11–14% in mainland populations. If we exclude the loci associated with the third-chromosome gene arrangements, then, with the single exception of *Pt-8*, the most common or only allele in Bogotá is the one in highest frequency in the mainland populations. More recent genetic analysis of fresh population samples from Charleston Mountains (Nevada), Wild Rose in the Panamint Mountains (California), Cerbat Mountains (Arizona), eastern Colorado and Guatemala, shows that all these populations are genetically very similar and resemble the mainland populations of Strawberry Canyon, Mesa Verde and Austin.

The genetic changes that have occurred in the Bogotá population are most likely due to founder effect, inbreeding and isolation from the main body of the species. The Bogotá population is completely isolated from the main body of *D. pseudoobscura* which extends into Guatemala. All attempts by members of the Genetics group of the University of Texas to collect this species from Costa Rica

and Panama have been unsuccessful (WHEELER personal communication). The Bogotá population has the lowest frequency of lethal and semilethal chromosomes observed in any *D. pseudoobscura* population (DOBZHANSKY *et al.* 1963; MAYHEW *et al.*, 1966) and the frequency of allelism of lethals is much greater in the Bogotá population than in the Guatemalan population (*MAYHEW et al.* 1966). Furthermore, there are only two gene arrangements, Santa Cruz and Tree Line, present in Colombian populations. The Guatemalan population has gene arrangements Oaxaca and Cuernavaca in addition to Santa Cruz and Tree Line arrangements. These facts strongly suggest that the Bogotá population was started by replication of a few chromosomes and that there has been considerable inbreeding in this population in the recent past. Genetic analysis of 24 loci adds further support to the argument that the Bogotá population was started by a few founders and has undergone considerable inbreeding.

From the results reported in this paper, we note that there is no sexual isolation between various mainland and Bogotá populations; we observe complete random mating between flies of different populations (Table 1). However, the Bogotá population has acquired one way cross sterility of F_1 hybrid males. The Bogotá population thus provides an example of a geographic isolate in the process of speciation. One way F_1 male sterility plus the loss of viability in backcross males shows that considerable reproductive isolation has developed in this population. Our results provide support to the argument that in most cases cross sterility is acquired in geographically isolated populations. When cross sterile populations become sympatric, then there will be selection for acquisition of additional isolating mechanisms such as sexual isolation, etc. (MAYR 1963; DOBZHANSKY 1970). Drosophila species of the *obscura* group provide a nice example for reconstructing the whole speciation phenomenon. We observe one-way male sterility in hybrids of allopatric Bogotá and mainland *D. pseudoobscura* populations but no sexual isolation. The sibling species *D. pseudoobscura* and *D. persimilis* which are sympatric over a large part of their distribution range, always produce sterile F_1 males; they do not mate in nature and in laboratory experiments strong sexual isolation is observed between them (MAYR and DOBZHANSKY 1945). The species *D. pseudoobscura*, *D. persimilis* and *D. miranda* occur sympatrically and the crosses between *D. miranda* and *D. pseudoobscura* or *D. persimilis* produce sterile hybrids of both sexes; the sexual isolation between these species is very strong (DOBZHANSKY *et al.* 1968). Here then, we observe a progression of events from one-way hybrid male sterility and no sexual isolation in crosses of Bogotá and mainland *D. pseudoobscura* to complete F_1 male sterility and the development of sexual isolation in crosses of *D. pseudoobscura* and *D. persimilis* and to sterility of both sexes plus very strong sexual isolation in crosses of *D. miranda* with *D. peudoobscura* or *D. persimilis.*

The development of reproductive isolation is the crux of speciation. Various hypotheses have been advanced as explanations for the origin of reproductive isolation. The most widely accepted idea long held by systematists is that isolating mechanisms arise as an incidental by-product of genetic divergence for environmental adaptations in isolated populations. MAYR (1963, p. 551) states "The

ecological shifts in incipient species are bound to have an effect on their isolating mechanisms. The thesis of the origin of reproductive isolation as a by-product of total genetic reconstitution of the speciating population is consistent with all the known facts." According to DOBZHANSKY (1970, p. 379) "Post mating isolating mechanisms (i.e., hybrid inviability, sterility, breakdown, or combination of these) are, then, consequences of differential adaptedness of races or species to the conditions of life in their respective distribution areas. They are by-products of genetic divergence. . . ." Our analysis of Bogotá population provides information on two important aspects of the origin of reproductive isolation—the magnitude of genetic changes responsible for development of reproductive isolation and the mode of origin of these genetic changes. Do geographically isolated populations develop reproductive isolation due to genetic changes at a large number of loci which adapt the organisms to their local environment or can we offer an alternative explanation? We will conclude that reproductive isolation can arise in a geographic isolate without incorporation of different alleles at many loci and the genetic changes responsible for origin of reproductive isolation can arise due to founder effect and inbreeding in the geographic isolate.

Even though gene frequency changes have occurred at most of the loci in the Bogotá population, there is not a single locus, out of 24 loci that have been studied, which has a different allele than that found in mainland (Table 7). The only case of a different allele reported in PRAKASH *et al.* (1969) was of $Pt\text{-}13^{1.37}$, but recently we have found this allele in mainland populations. Furthermore, the hybrid male sterility can be explained by differentiation of the Bogotá population at only four genetic loci, two on the *X* chromosome and two on the autosomes. We must conclude then that reproductive isolation can arise without a large

TABLE 7

A. Gene differences between Bogotá (Colombia) and mainland *D. pseudoobscura* populations from various localities in the United States and from Guatemala (from Prakash, Lewontin and Hubby, 1969 and unpublished work).	
1. Number of unique alleles in Bogotá/total alleles in all populations	0/64 (0.0)*
2. Number of loci with only unique allele(s) in Bogotá/total loci studied	0/24 (0.0)
B. Gene differences between *D. pseudoobscura* populations from mainland United States and Guatemala and *D. persimilis* (from Prakash, 1969 and Prakash, Lewontin and Hubby, 1969) and unpublished work.	
1. Number of unique alleles in *D. persimilis*/total alleles in both species	7/71 (0.098)
2. Number of loci with only unique allele(s) in *D. persimilis*/total loci studied in both species	0/24 (0.0)

* Numbers in parenthesis are the proportions.

amount of genetic change in the geographic isolate. Even the comparison of sibling species *D. pseudoobscura* and *D. persimilis* (Table 7) shows that while 12% of the alleles in *D. persimilis* are unique, i.e., they do not occur in *D. pseudoobscura*, there is not a single locus in *D. persimilis* which has only unique allele(s). Structural genes can thus remain remarkably stable during speciation.

We can only speculate about the manner in which genetic changes responsible for hybrid male sterility might have arisen in the Bogotá population. While it is probable that genetic changes which are responsible for local environmental adaptation have incidentally given rise to reproductive isolation, it is more likely that reproductive isolation evolved in the Bogotá population as a result of founder effect and inbreeding. HOLLINGSWORTH and MAYNARD SMITH (1955) found that inbreeding in *D. subobscura* leads to a major increase in male infertility which is due to inadequacy of a proportion of sperms produced by these males. It is reasonable to assume that founder effect and inbreeding in the Bogotá population led to a reduction in fertility of males. Any genetic changes which improve male fertility would have been incorporated in the Bogotá population. Fixation of different allele(s) at a few loci in Bogotá which improve male fertility might have led to the observed incompatibilities between Bogotá and mainland chromosomes with the result that considerable reproductive isolation has developed in Bogotá. According to this view, reproductive isolation evolved in the Bogotá population of *D. pseudoobscura* as a consequence of founder effect and inbreeding and not as a by-product of genetic divergence which is presumed to occur as an adaptive response to local environment.

The probable age of the Bogotá population merits consideration in order to understand the time period involved in the development of reproductive isolation. From 1955–1960, extensive collections of Drosophila species were made in Colombia by the Genetics Group from The University of Texas at Austin. The places where collections were made varied from north to south in Colombia and from sea level to mountains higher than Bogotá itself, but no *D. pseudoobscura* were caught in these collections. However, in August 1960, Dr. ALICE HUNTER had *D. pseudoobscura* strains in her laboratory which were collected from traps outside her laboratory (WHEELER, personal communication). Since there had been no *D. pseudoobscura* in Dr. HUNTER's laboratory before this time, there was no possibility of *D. pseudoobscura* being released from her laboratory.

D. pseudoobscura in Colombia is confined to a central region of the Eastern Cordillera of the Andes. The species has been collected from localities 50 or fewer kilometers from the city of Bogotá except Paipa, which is 225 kilometers north of Bogotá. In these areas, this species is encountered at elevations of 2,200 to 3,280 meters. The species is locally common and constitutes between one and 55% of the caught Drosophila species. This species has not been found in collections from Pasto, San Lorenzo, Manizales and Medellin (DOBZHANSKY *et al.* 1963; HUNTER 1966). These two observations, *viz.*, first, *D. pseudoobscura* was never found in extensive collections from 1955–1960 around the city of Bogotá and from the rest of Colombia and second, *D. pseudoobscura* has been found to occur in restricted areas of Colombia, mainly around the city of Bogotá, make it highly probable

that the population in Colombia is of very recent origin. The exact age of the population can never be established precisely but what is significant for us is that apparently reproductive isolation can develop in an isolate in a very short time.

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LITERATURE CITED

ANDERSON, W. W. and L. EHRMAN, 1969 Mating choice in crosses between geographic populations of *Drosophila pseudoobscura*. Am. Midland Naturalist **81**: 47–53.

DOBZHANSKY, TH., 1970 *Genetics of the Evolutionary Process*. Columbia University Press. New York.

DOBZHANSKY, TH., A. S. HUNTER, O. PAVLOVSKY, B. SPASSKY and B. WALLACE, 1963 Genetics of natural populations. XXXI. Genetics of an isolated marginal population of *Drosophila pseudoobscura*. Genetics **48**: 91–103.

DOBZHANSKY, TH., L. EHRMAN and P. A. KASTRITSIS, 1968 Ethological isolation between sympatric and allopatric species of the *obscura* group of Drosophila. Animal Behavior **16**: 79–87.

HOLLINGSWORTH, M. J. and J. MAYNARD SMITH, 1955 The effects of inbreeding on rate of development and on fertility in *Drosophila subobscura*. J. Genet. **53**: 295–314.

HUNTER, A. S., 1966 High altitude Drosophila of Colombia. (Diptera: Drosophilidae). Ann. Ent. Soc. Amer. **59**: 413–423.

MAYHEW, S. H., S. K. KATO, F. M. BALL and C. EPLING, 1966 Comparative studies of arrangements within and between populations of *Drosophila pseudoobscura*. Evolution. **20**: 646–662.

MAYR, E., 1963 *Animal species and evolution*. The Belknap Press of Harvard University Press. Cambridge, Mass.

MAYR, E. and TH. DOBZHANSKY, 1945 Experiments on sexual isolation in Drosophila. IV. Modification of degree of isolation between *Drosophila pseudoobscura* and *Drosophila persimilis* and of sexual preferences in *Drosophila prosaltans*. Proc. Natl. Acad. Sci. U.S. **31**: 75–82.

PRAKASH, S., 1967 Chromosome interactions in *Drosophila robusta*. Genetics **57**: 385–400. ——, 1969 Gene variation in a natural population of *Drosophila persimilis*. Proc. Natl. Acad. Sci. U.S. **62**: 778–784.

PRAKASH, S. and R. C. LEWONTIN, 1968 A molecular approach to the study of gene heterozygosity in natural populations. III. Direct evidence of Coadaptation in gene arrangements of Drosophila. Proc. Natl. Acad. Sci. U.S. **59**: 398–405. ——, 1971 A molecular approach to the study of gene heterozygosity in natural populations. V. Further direct evidence of Coadaptation in inversions of Drosophila. Genetics **69**: 405–408.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY, 1969 A molecular approach to the study of gene heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics **61**: 841–858.

STURTEVANT, A. H. and TH. DOBZHANSKY, 1936 Geographical distribution and cytology of "sex ratio" in *Drosophila pseudoobscura* and related species. Genetics **21**: 473–490.