

for this system, as is observed. In the TpT plus purine system, there is no preferential interaction between the incorporated purine with either base of the dinucleotide. The linewidths of the purine resonances should therefore be intermediate between those for TpdU and dUpT. In fact, the widths of the H₆ and H₈ proton resonances are close to the mean of the widths for TpdU and dUpT. Similar line-broadening results have now been obtained for BdUpT, ApU, and UpA, which appear to be consistent with this model. (BdU = 5-bromo-2'-deoxyuridine, A = adenosine, and U = uridine.) For example, the purine proton resonances are sharp in UpA; but in BdUpT and ApU, they are so broad as to be unobservable for purine/dinucleotide ratios less than 2:1. On the basis of the larger self-association tendency of 5-bromouridine compared to uridine and thymidine,² purine is expected to interact more strongly with the 5-bromouracil base of BdUpT; it is also expected to interact preferentially with the adenosine base in UpA and ApU. Since the mean lifetime of the purine incorporated in these systems is expected to be longer than that in TpdU, dUpT, and TpT, the broadening of the purine proton resonances should be more pronounced, as observed.

The specificity of the line-broadening suggests that the orientation of the inserted purine molecule relative to the dinucleotide is not completely random. The larger line-broadening observed for H₆ and H₈ protons indicates that there may be some preference for the purine base to be oriented with these protons directed at the ribose protons of the 3'-nucleoside.

Similar studies on other dinucleotides (ApA, ApU, UpA, UpU, CpC, CpA, and ApC, where C = cytidine) have also been completed within our laboratories. These results will be published in detail elsewhere.

* Alfred P. Sloan fellow.

† National Science Foundation predoctoral fellow.

‡ Contribution no. 3321.

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SPONTANEOUS ORIGIN OF AN INCIPIENT SPECIES IN THE DROSOPHILA PAULISTORUM COMPLEX*

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It has been questioned, by His Holiness Pius XII¹ among others, whether biology has really succeeded in making a species from another species. Fertile allopolyploids derived from hybrids between species have all the properties of new species. The clinching argument is that not only have new species been obtained

in this way but also some species existing in nature have been resynthesized. Species formation through doubling of the chromosomal complement in a hybrid is, however, not the usual method of speciation, though it is common enough in certain families of plants. A more general way, among sexually reproducing and cross-fertilizing animals and plants, is through construction of reproductive isolating mechanisms which impede or eliminate the gene exchange between genetically diverging populations. This process has been inferred to have taken place in numerous examples, but it is generally too gradual and slow to be observed directly. An exceptional situation, the occurrence in a laboratory line of a first step toward hybrid sterility, is reported in the present article.

Incipient Species in Drosophila paulistorum.—The superspecies *Drosophila paulistorum* (Dobzhansky and Pavan) is a complex of six arrays of Mendelian populations which are too distinct to be considered races of the same species but not distinct enough to be regarded full species.^{2, 3} As a compromise we have called them “races or incipient species.” They show various degrees of sexual (ethological), isolation, since the females accept males of their own but usually reject those of the other incipient species. If insemination does occur, hybrid offspring is produced, consisting of fertile daughters and completely sterile sons. Only some strains of the Transitional race from northern and western Colombia cross and give fertile progenies with more than a single incipient species. Among the backcross progenies, the males remain sterile as long as their mothers carry one or more chromosomes of a foreign species; the acceptability of an individual as a mate depends, however, simply on the prevalence in the potential mates of the genes derived from one or another of the incipient species.^{4, 5}

The incipient species are morphologically indistinguishable. To diagnose an unknown strain, it is crossed to five “testers” belonging, respectively, to the Centrocuban, Orinocan, Amazonian, Guianan, and Andean-Brazilian incipient species.^{2, 3} The result is usually unambiguous, since fertile hybrids are produced with one and only one tester strain.

A Strain Changing Its Genetic Behavior.—On March 19, 1958, a sample of *Drosophila* was collected at Chichimene, south of Villavicencio, in the Llanos of Colombia. A strain called “Llanos-A” was derived from the progenies of several females which intercrossed freely and gave fertile hybrids. It was classified as a member of the Orinocan incipient species, because it gave fertile hybrids with most Orinocan strains, and despite somewhat variable behavior with other strains.² After repeated testing it did, however, yield fertile hybrids with every Orinocan strain then available. A cytological study of the giant chromosomes in the larval salivary glands showed several inversions not found in any other strain.⁶ There is nothing unusual in this, because endemic chromosomal polymorphs occur also in some other strains of *D. paulistorum*.

About 5 years later, in 1963, new population samples were taken in several localities in Venezuela, Trinidad, and British Guiana. In classifying these, the Llanos-A strain was employed as a tester for the Orinocan incipient species. Several strains from Trinidad and British Guiana gave sterile hybrids with Llanos-A and the other testers, although they were fully fertile *inter se*. The conclusion was drawn that these strains belonged to a new, seventh, incipient species which was named “Calypso.”³ Later in the same year and in 1964, numerous crosses between

TABLE 1
CROSSES OF THE LLANOS-A STRAIN WITH OTHER INCIPIENT SPECIES

Crossed to:	Llanos-A ♀♀		Llanos-A ♂♂	
	0*	S*	0*	S*
Centro-American	1	5	—	9
Transitional	4	2	9	1
Orinocan	—	22	—	23
Amazonian	8	—	17	2
Guianan	1	1	5	—
Andean-Brazilian	1	3	6	7

* 0 = no progeny; S = sterile F₁.

strains of all incipient species were being made for another purpose. The Llanos-A strain now behaved in a totally unexpected manner; namely, it failed to give fertile hybrids with any strain other than itself, including Orinocan strains with which it produced fully fertile hybrids about 4 years earlier. The supposed Calypso strains, however, crossed freely and gave fertile hybrids with all other Orinocan strains except Llanos-A. It became clear that Calypso is nothing but a part of the Orinocan incipient species. On the other hand, Llanos-A now behaved as a new and hitherto unknown incipient species.

The situation raised a suspicion that the Llanos-A may have been accidentally contaminated with some other strain. This can be ruled out on two grounds. First, *D. paulistorum* occurs in New York only in our laboratory, and no other strain behaved like Llanos-A. Second, a cytological examination disclosed that the strain still carried all the unique karyotypes which were found in it when it behaved like a "normal" Orinocan strain. The problem now is to investigate the properties of the new, spontaneously arisen, incipient species.

Hybrid Sterility in Crosses of Llanos-A with Other Strains.—The results of the crosses carried out in 1964 and 1965 between Llanos-A and other strains are summarized in Table 1. The sign 0 means no progeny was obtained, and S that F₁ but no F₂ hybrids were produced, because of the sterility of the F₁ hybrid males. No fertile crosses at all were observed.

The Llanos-A strain is most easily crossable to Orinocan strains, but the F₁ progenies produced fail to give F₂ progenies, because of the sterility of the males. With the other incipient species, there is either no progeny, or an F₁ with male sterility. The causation of this sterility must now be examined. As shown by Ehrman,^{4,7} the sterility of the F₁ is different from that of the backcross hybrid males. These latter are sterile even if all their genes are those of one species, provided only that their mothers carried at least one foreign chromosome. Yet the F₁ hybrids come from nonhybrid mothers, so that their sterility must be caused by their hybrid genome.

Fertility and Sterility in the Backcrosses.—As stated above, in the hybrid progenies of the incipient species not only the F₁ but also the backcross males are sterile. In contrast to this, the backcross hybrids in the progenies of Llanos-A × Orinocan are mostly fertile. Experiments were made using the autosomal recessive mutant orange eyes, found by one of us (O. P.) in the Orinocan strain from Georgetown, British Guiana. Both reciprocal crosses, orange ♀ × Llanos-A ♂ and Llanos-A ♀ × orange ♂, gave sterile F₁ sons and fertile daughters. These latter were backcrossed to orange males. The backcross progenies showed the expected 1:1 segregation for orange and normal red. Fertility tests were made using groups of 6–

12 hybrid males mated to orange Georgetown Orinocan females. All crosses with orange-eyed backcross males were fertile. The red-eyed males gave variable results, no progenies in some and small amounts in other cultures. Dissection of the males showed apparently normal sperm in their testes and seminal vesicles. Second and third backcrosses were made, using both orange and red backcross females. Orange as well as red-eyed males in the BC₂ and BC₃ progenies seemed fully fertile.

The change which altered the properties of the Llanos-A did not make it genetically as distinct as the "natural" incipient species are from each other. Llanos-A does produce sterile F₁ hybrid males with Orinocan strains with which it was formerly fertile, but the backcross males are partially or even fully fertile. The question logically arises whether backcross males will also be fertile when the Llanos-A strain is crossed to incipient species other than the Orinocan. Crosses have consequently been made of Llanos-A to (1) the autosomal recessive mutant orange eye from a strain of Panamanian origin of the Amazonian incipient species, (2) autosomal recessive ebony, Lancetilla strain of Centro-American, and (3) autosomal recessive "dark eye" (possibly a homologue of brown), Apoteri strain of Andean-Brazilian incipient species. All these crosses yielded completely sterile F₁ male and fertile female hybrids; the backcrosses again gave fertile females and sterile males. A preliminary cytological study showed disturbances in the spermatogenesis, which tend to be even more drastic in the backcross than in the F₁ hybrid males. We hope to report these and related results in another publication.

Sexual Behavior of the "New" Incipient Species.—The incipient species of *D. paulistorum* found in nature are reproductively isolated by their sexual preferences, as well as by the sterility of the male hybrids between them. The ethological isolation and the hybrid sterility are due to two distinct genetic mechanisms.^{4, 5} Llanos-A, a former member of the Orinocan race or incipient species, is now giving sterile F₁ male hybrids with Orinocan strains. We wish to inquire whether or not Llanos-A became ethologically isolated concomitantly with having evolved a hybrid sterility.

Although members of the same incipient species are always and easily crossable to each other, a quantitative study sometimes detects relatively slight but still significant differences in the sexual preferences among strains of the same incipient species derived from different geographic areas. In 1958–1961, the strain Llanos-A was among the 25 strains which were tested for mating preferences by Carmody *et al.*⁶ The so-called "male-choice" method was used; groups of usually ten virgin females of each of two strains were confined with ten males of one strain for a time sufficient to have approximately half of the females inseminated; the females were then dissected and their sperm receptacles examined under a microscope for the presence of sperm. Statistically significant preferences for homogamic matings (i.e., females with males of the same strain) were detected in the following combinations of Llanos-A with other Orinocan strains:

<i>Females</i>	<i>Males</i>
Llanos-A + Panama	Panama
Llanos-A + Panama	Llanos-A
Llanos-A + Caripe	Llanos-A
Llanos-A + Guiana-B	Llanos-A

TABLE 2
NUMBERS OF INSEMINATED (+) AND UNINSEMINATED (-) FEMALES WHEN FEMALES
OF TWO STRAINS ARE EXPOSED TO MALES OF ONE STRAIN

Males	Females				Isolation index
	Llanos-A		Orange		
	+	-	+	-	
Llanos-A	56	34	36	54	+0.22
Orange	44	47	44	48	-0.06

TABLE 3
NUMBERS OF INSEMINATED (+) AND UNINSEMINATED (-) FEMALES IN TESTS OF LLANOS-A WITH
THE ANDEAN-BRAZILIAN (AB) AND CENTRO-AMERICAN (CA) STRAINS

Females	Males	Homogamic		Heterogamic		Isolation index
		+	-	+	-	
Llanos-A + AB	Llanos-A	26	2	1	26	+0.92
" "	AB	18	12	2	26	+0.79
" CA	Llanos-A	14	12	6	23	+0.44
" "	CA	24	5	5	25	+0.66

No mating preferences appeared in reciprocal combinations (e.g., Guiana-B males with Guiana-B and Llanos-A females). Although the "male-choice" method has certain drawbacks, we have used it to make the tests of the "new" Llanos-A strain comparable with the data of Carmody *et al.* Groups of ten virgin females of Llanos-A and of the orange mutant were kept for several hours with either ten Llanos-A or with ten orange males (Orinocan, Georgetown). The flies were aged for 5-7 days before the exposure. Ten replications were made. The results are reported in Table 2.

There is a small but significant isolation index in crosses with Llanos-A males, but random mating with orange males. The conclusion that in developing a hybrid sterility with Orinocan strains the Llanos-A strain has also not developed an ethological isolation against them is made inescapable by the behavior of Llanos-A with other incipient species. We have tested it by "male-choice" method with the mutant dark eyes (possibly homologue of brown) of the Andean-Brazilian, and with ebony of the Centro-American incipient species. The technique was the same as in the tests with the orange (Orinocan) mutant, except that only three replications were run. Table 3 reports the results.

The isolation indexes are all positive and statistically highly significant (an index 1.0 would mean that the isolation is complete). Whatever change has taken place in the Llanos-A strain, it has led neither to establishment of an ethological isolation from Orinocan strains, nor to a breakdown of the isolation from the other incipient species. The isolation with the Andean is stronger, however, than with the Centro-American incipient species, which may be related to the fact that both Orinocan and Andean, but no Centro-American flies, were found in the Llanos locality.

Conclusions.—Llanos-A is a new race or an incipient species having arisen in the laboratory at some time between 1958 and 1963. It has diverged from its progenitor, Orinocan; less than the five naturally existing Orinocan, Amazonian, Centro-American, Guianan, and Andean-Brazilian incipient species have diverged from each other. (The Transitional race is only a marginal variant of the Andean-Brazilian.⁶) There is neither ethological isolation nor a complete sterility of the male hybrids in the backcrosses of Llanos-A with Orinocan strains. The sterility of the F₁ male hybrids is, however, complete.

The nature of the change which has transformed Llanos-A strain is conjectural.

This genetic change is accompanied neither by a morphological alteration nor by a change in the gene arrangement visible in the chromosomes of the larval salivary glands. An interesting, though entirely hypothetical, possibility is suggested by the experiments of Ehrman and Williamson⁹ on Mesitas and Santa Marta strains of the Transitional race of *D. paulistorum*. The cross Santa Marta ♀ × Mesitas ♂ yields sterile male and fertile female hybrids, while the reciprocal cross gives fertile hybrids of both sexes. Ehrman and Williamson injected Mesitas females with homogenates of the hybrid flies or of those of the Santa Marta strain. The injected females were crossed to Mesitas males; the progenies had evidently only Mesitas genes, and should have been fertile. Yet the males which developed from the eggs deposited by their mothers a week or more after the injection were sterile. Some factor provoking hybrid sterility can evidently be introduced by injection as well as by crossing. What this factor is remains uncertain; but an infection with a symbiont or a commensal genetically adapted in the Santa Marta but not in the Mesitas strain is strongly suggested.

Another, and as yet unpublished, result of Williamson and Ehrman, which they kindly permit to be mentioned here, pertains to the Llanos-A strain. They injected homogenates of sterile hybrids of Llanos-A with an Amazonian strain from Panama into Mesitas and other females, attempting to induce a sterility in the male progeny. The injection proved fatal, or nearly so, to Mesitas females; in a control experiment, Mesitas females were injected with homogenates of strains other than Llanos-A and survived. Perhaps, then, the extraordinary behavior of the Llanos-A strain is due to its having acquired and become adapted to a new commensal or symbiont.

The behavior of Llanos-A is not quite unique. The change in this strain occurred after it was bred for some generations in the laboratory. We have evidence suggesting that changes of this sort can occur in nature as well. Dr. S. B. Pipkin kindly sent us a collection of *D. paulistorum* from Rio Raposo, on the Pacific Coast of Colombia. These flies gave strains which failed to yield fertile F₁ hybrid males with any other strain in our laboratory. Does Rio Raposo belong to a new incipient species? Experiments with the Rio Raposo strains, to be reported in another publication, disclosed that they stand in a relationship with the Centro-American incipient species similar to that between Llanos-A and its Orinocan progenitor. Hybrid sterility, and consequently incipient reproductive isolation, evidently arises from time to time within the superspecies *D. paulistorum*.

Summary.—The superspecies *Drosophila paulistorum* is a complex of several incipient species. Strains of the same incipient species cross easily and produce fertile hybrids; strains of the different ones exhibit strong ethological (sexual) isolation, and the F₁ hybrids are fertile females and sterile males. The male sterility continues in the backcrosses.

A strain established in the laboratory from flies collected in the Llanos of Colombia in 1958 behaved at first as a member of the Orinocan incipient species. By 1963 it changed so that its male F₁ hybrids with other Orinocan strains became completely sterile. The backcross hybrids, however, continue fertile, and no strong ethological isolation from Orinocan strains has arisen. The reproductive isolation between Llanos and other incipient species has not been altered. Some possible causes of the emergence of the new incipient species are indicated.

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ON THE INFLUENCE OF NATURAL SELECTION ON POPULATION SIZE

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1. *Introduction.*—We are concerned with a problem first considered by J. B. S. Haldane¹ and by M. Kimura.² These authors introduced a measure for the loss in population size caused by a slow natural selection; this loss is called selective death by Haldane, and substitutional (or evolutionary) load by Kimura. Their calculations lead to the conclusion that the presumed loss may become so enormous as to preclude a simultaneous selection at several loci at a reasonable rate. My attention was drawn to these calculations by Th. Dobzhansky who explained to me the grave consequences of such a result. Other eminent biologists were worried by Haldane's conclusions.³

Fortunately for modern evolution theory, the calculations turn out to contain an error which invalidates the result. As has been pointed out many times, the application of the familiar formulas for gene frequencies to natural populations presupposes that the total population size remains constant. A changing population size introduces an inaccuracy which is in practice negligible over reasonably short periods, but the error is cumulative and cannot be neglected in calculations involving long periods of time. This point is of considerable importance because it is customary in evolution theory to think exclusively in terms of relative frequencies and relative fitnesses with a complete neglect of population sizes and absolute rates of change. The dangers of this habit are discussed in a paper⁴ which contains also a detailed analysis of Haldane's calculations. The object of the present paper is to present the solution of Haldane's problems in an interesting special case.

2. We consider the selection at one autosomal locus in a sexually reproducing diploid without mutations under random mating. We associate with the three genotypes *AA*, *Aa*, and *aa* absolute fitnesses w_1 , w_2 , and w_3 . In other words, w_1 is the expected number of offspring (counted at an appropriate stage of development) resulting from the pairing of two *A*-alleles. Then