COMPLETE REPRODUCTIVE ISOLATION BETWEEN TWO MORPHOLOGICALLY SIMILAR SPECIES OF DROSOPHILA

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The magnitude of morphological differences between related species is variable. Species of some genera differ in appearance strikingly enough to be easily distinguishable to a layman; in other genera the differences are recondite and can be detected only by specialists. The limiting case is, evidently, when two or more species are nearly or completely identical in morphology. Thanks chiefly to the work of Patterson and his school, it is now clear that Drosophila is a genus remarkably rich in pairs and groups of morphologically very similar species. Thus, Patterson and Wheeler ('42), Patterson ('43), and Patterson and Mainland ('44) have been able to distinguish 28 species of the *repleta* group of Drosophila, while Duda ('25) considered this group to contain a single one. In fairness to Duda, it must be stated that he worked exclusively with pinned museum material.

To an ecologist or a geneticist, morphologically similar species are important because their existence proves that morphological differentiation is not an essential, though widespread, concomitant of evolutionary divergence. The present article reports a particularly interesting case of this kind: *Drosophila willistoni* Sturtevant and *Drosophila equinoxialis* Dobzhansky show a complete reproductive isolation contrasting sharply with a virtual lack of morphological differences.

THE WILLISTONI SPECIES GROUP

Species of the *willistoni* group of the subgenus Sophophora of the genus Drosophila occur in the tropical and subtropical Americas. Apart from *D. willistoni* and *D. equinoxialis,* this group includes also *D. paulista* Dobzhansky and Pavan, a South-Brazilian species morphologically close to but not identical with the former two, *D. capricorni* Dobzhansky and Pavan in South Brazil and its Mexican relative *D. sucinea* Patterson and Mainland, *D. fumipennis* Duda, the more distantly related *D. nebulosa* Sturtevant, and possibly *D. subinfumata* Duda.

Sturtevant ('21), Duda ('25), Patterson and Wagner ('42), Patterson and Mainland ('43), and Dobzhansky and Pavan ('43) record the occurrences of of *D. willistoni* in southern Florida; Nassau in the Bahamas; Cuba, Haiti, Porto Rico, St. Vincent, and Jamaica; states of Coahuila, Tamaulipas, San Luis Potosi, Vera Cruz, Morelos, Guerrero, Michoacan, and Jalisco in Mexico; Costa Rica and Panama; Manaos, Rio de Janeiro, and state of São Paulo in Brazil; and Mapiri in Bolivia. Several findings in the vicinity of Belem do Para, Brazil, may be added to these records. Thus, *D. willistoni* is widespread in the American tropics, with the possible exception of the West Coast of South America. In southern Brazil and in Para, it is also one of the commonest species of Drosophila. At any rate, the writer frequently found hundreds and even thousands of specimens hovering on various decaying fruit (particularly *Ficus* sp.), both in the jungle and in the cultivated zones, although in the latter it is exceeded in frequency by *D. ananassae* Doleshall and *D. simulans* Sturtevant, scavengers now found apparently throughout the wet Tropics *D. equinoxialis* Dobzhansky is known only from Teffe, state of Amazonas, Brazil.

SEXUAL ISOLATION

The writer has at his disposal five living strains of *D. willistoni* derived from Praia Grande and Bertioga, state of São Paulo, from Rio de Janeiro, from Belem do Para,

Brazil; and from Quirigua, Guatemala. The single strain of *D. equinoxialis* came, as stated, from Teffe, Brazil. The Brazilian strains are each descended from several wild females and males collected in the localities named above by Dr. C. Pavan and the writer. The Quirigua strain, obtained through the courtesy of Prof. A. H. Sturtevant, is descended from an unknown number of wild progenitors. When collected and examined at Teffe, as well as when re-examined in New York, the Teffe strain failed to attract attention and was classified as *D. willistoni*. Professor J. T. Patterson kindly consented to examine this strain, and classified it as belonging to the the same species.

The above six strains were used by Dobzhansky and Mayr ('44) for experiments on sexual isolation. The technique consisted in placing ten males of a given strain together with ten virgin females of the same strain and ten females of a different strain. Such batches of thirty flies were kept together in vials with food for about two days at 25° C., whereupon all the females were dissected and their sperm receptacles examined under the microscope for presence or absence of sperm. With the exception of the Teffe strain, all strains intercrossed freely. Brazilian males from the Praia Grande, Bertioga, Rio, and Belem strains inseminate as many females of their own strains as they do of females of other Brazilian strains. When either Guatemala (Quirigua) or Brazilian males are placed together with a mixture of Guatemala and Brazilian females, slightly but significantly more of the latter than of the former are inseminated. (For details see Dobzhansky and Mayr '44.)

The strikingly different results which were obtained when the Teffe (*D. equinoxialis*) strain was used are summarized in table I. Teffe males inseminate almost exclusively Teffe females, just as *D. willistoni* males accept only females of their own species. Although in these experiments the flies were kept together in vials for up to five days, Teffe males have inseminated only one out of 257 *D. willistoni* females and 204 out of 266 females of their own species. *D. willistoni* males have inseminated none out of 73 Teffe females and 46 out of 71 females of their own species.

TABLE I. *Females dissected (n) and females found inseminated (s) in mixtures of females of the Teffe strain of Drosophila equinoxialis and of some strains of Drosophila willistoni*

Female strains	Male strain	*D. willistoni*		*D. equinoxialis*	
		s	n	s	n
Praia Grande, Teffe..	Teffe	1	78	68	81
Bertioga, Teffe.....	Teffe	—	61	36	64
Rio, Teffe.....	Teffe	—	70	58	72
Belem, Teffe.....	Teffe	—	28	27	30
Quirigua, Teffe.....	Teffe	—	19	15	19
Teffe, Belem.....	Belem	28	51	—	53
Teffe, Rio.....	Rio	18	20	—	20

Sexual isolation between *D. equinoxialis* (Teffe) and *D. willistoni* undoubtedly exists. To see just how strong it is, experiments were made in which the flies could mate either interspecifically or not at all. Groups of from 30 to 50 females of a given strain of one species were placed with an equal or greater number of males of the other species in regular culture bottles with food, and kept together for from 6 to 47 days at summer room temperature. The assistance of Mrs. N. P. Dobzhansky and of Miss Irene Markreich in this phase of the experiments is gratefully acknowledged. From time to time, as the food in the bottles was becoming unsatisfactory to the flies, the survivors were transferred to fresh bottles. The females were finally dissected and examined for sperm in their receptacles. The results obtained are summarized in table II.

The mutual repulsion between the two species is obviously very strong. *D. willistoni* males inseminated only about 1.5 per cent of the Teffe females even when confined with them and no other females for more than a month. *D. equinoxialis* (Teffe) males inseminate about 4 per cent of *D. willistoni* females, and there is an

TABLE II *Females dissected (n) and females found inseminated (s) in experiments in which no choice of species for mating was possible*

D. equinoxialis ♀♀ × *D. willistoni* ♂♂				*D. willistoni* ♀♀ × *D. equinoxialis* ♂♂			
Male strain	Days together	n	s	Female strain	Days together	n	s
Praia Grande	7	38	1	Praia Grande	6	47	1
Bertioga	7	24	—	Bertioga	6	37	—
Belem	11	53	—	Rio	6	110	3
Rio	11	60	1	Belem	6	38	—
Quirigua	11	29	—	Quirigua	6	33	—
Belem	12	29	3	Belem	21	29	—
Quirigua	30	50	1	Praia Grande	25	27	2
Bertioga	31	18	—	Bertioga	31	34	1
Belem	32	29	—	Rio	31	35	1
Bertioga	36	33	—	Praia Grande	33	64	4
Rio	36	24	—	Belem	34	78	6
Belem	36	10	—	Bertioga	35	44	1
Quirigua	36	34	1	Praia Grande	38	15	1
Bertioga	43	15	—	Rio	38	71	2
Rio	43	29	—	Belem	41	67	6
Quirigua	43	30	—				
Bertioga	45	26	1				
Bertioga	47	14	—				
Total		545	8	Total		729	28

indication that old males may be slightly less discriminating than young ones.

NON-PRODUCTION OF HYBRIDS

Since *D. equinoxialis* and *D. willistoni* do, though very rarely, mate, care was taken in the just reported experiments on sexual isolation to detect any hybrids that may be produced. Virgin Drosophila females eventually deposit their unfertilized eggs; the surface of the food in the bottles in which males of one species are kept together with females of the other soon becomes covered with eggs. However, in all the bottles but three, no trace of larvae has appeared. It is evident that the females inseminated by males of the other species deposit eggs that are either unfertilized or inviable.

There is no doubt that experimental mistakes are responsible for the three exceptional bottles. As shown below, *D. willistoni* and *D. equinoxialis* begin to mate sooner after the hatching from the pupae than do most species of Drosophila. The problem of obtaining large numbers of virgins is, therefore, difficult. Although the bottles in which the flies were hatching were emptied at intervals of several hours, a few of the females were not virgin. The three bottles referred to above had fair numbers of larvae each, and the parents continued to deposit fertile eggs when transferred on fresh food. That the adult flies which hatched in these bottles were not hybrids is shown not only by their normal appearance but also by the fact that they were fertile and produced large F_2 progenies when inbred. The conclusive evidence is that when these flies were crossed to the presumed parental species, the crosses to one of the parents (*D. willistoni* twice, *D. equinoxialis* once) went without slightest difficulty, while those to the other parent did not go at all. The three bottles which produced larvae are not included in the material in table II.

BODY SIZE

The behavior of the Teffe strain shows clearly that it belongs to a species different from *D. willistoni*. A careful comparison has been made of the external morphological as well as of the anatomical characters in the strains available. As shown in table III, the body size proved to be smaller in

TABLE III. *Length of the body (in mm) in D. willistoni and D. equinoxialis*

Strain	Females			Males		
	M ± m	σ	n	M ± m	σ	n
Praia Grande	2.25 ± 0.03	0.12	20	1.93 ± 0.02	0.10	20
Bertioga	2.19 ± 0.03	0.12	20	1.91 ± 0.02	0.09	20
Rio	2.22 ± 0.03	0.13	20	1.88 ± 0.02	0.07	20
Belem	2.20 ± 0.03	0.14	20	1.95 ± 0.02	0.09	20
Quirigua	2.21 ± 0.03	0.111	20	1.87 ± 0.02	0.10	20
Total *willistoni*	2.21 ± 0.01	0.13	100	1.91 ± 0.01	0.09	100
equinoxialis, Teffe	1.98 ± 0.01	0.09	100	1.79 ± 0.01	0.07	100

D. equinoxialis than in *D. willistoni*. Very minor differences possibly exist also in the color of the antennae and in the relative lengths of the anterior and the posterior orbital bristles (see the description of *D. equinoxialis* below, and that of *D. willistoni* in Dobzhansky and Pavan '43). Apart from this, the two species are morphologically alike. The absence of differences in the external genitalia of the males deserves particular mention. The metaphase chromosome patterns are alike. *D. equinoxialis* is not favorable for the study of chromosomes in the salivary gland cells.

The material for the measurements of the body size was prepared as follows. Small groups of females and males of each strain were allowed to oviposit in usual culture bottles for about 24 hours. The bottles with the eggs were placed at 25° C. When the flies hatched they were transferred to bottles with fresh food for a few days to harden, whereupon the body length was measured in etherized flies as recommended by Dobzhansky and Pavan ('43). In *D. equinoxialis*, 100 females and 100 males were measured; 20 females and 20 males were measured in each of the five strains of *D. willistoni*. Table III shows little significant heterogeneity between the strains of *D. willistoni*. *D. equinoxialis* flies are undoubtedly smaller than those of the former species. The variation curves are, however, broadly overlapping, even in flies raised under uniform conditions. There is no possibility of determining the species of an average individual by its size.

SEXUAL MATURITY

A chance observation suggested that *D. willistoni* flies begin to copulate somewhat earlier after hatching from the pupae than do *D. equinoxialis*. To establish this point, approximately equal numbers of females and males of the same species were kept in bottles in an incubator at 25° C., and from time to time some of the females were dissected to determine what proportion of them were inseminated. When placed together, the flies were not older than 4 hours from the hatching from the pupae; their average age at that time may

TABLE IV. *Numbers of females dissected (n) and percent found inseminated (%) among flies of different age*

Age in hours	*D. equinoxialis*		*D. willistoni*	
	n	%	n	%
6	20	0	20	0
9	—	—	34	3
12	—	—	25	16
18	14	0	38	29
21	—	—	68	48
24	30	3	55	65
28	—	—	47	68
33	—	—	42	62
36	24	12	20	80
42	14	28	21	95
48	41	56	—	—
54	42	55	—	—
60	40	50	—	—
72	40	47	—	—
96	40	87	—	—

be taken to be 2 hours. The results are shown in table IV.

It can be seen that some 9 hours old *D. willistoni* flies had already copulated, while the first inseminations in *D. equinoxialis* are recorded in 24 hours old flies. At 24 hours, about half of *D. willistoni* females contain sperm, while in *D. equinoxialis* this proportion of inseminated females is reached at 48 hours.

A description of *D. equinoxialis,* made according to the form now accepted in Drosophila systematics, follows:

DROSOPHILA EQUINOXIALIS, SPECIES NOVA

♂, ♀. Arista with 10–11 branches. Antennae tan, third segment densely but delicately pilose. Front yellow. Anterior and posterior orbitals about equally long, middle orbital ¼ posterior. Two prominent orals. Face yellow. Carina short, gradually falling off below, not sulcate. Cheeks pale yellow, their greatest width about ⅙ greatest diameter of eye. Eyes cinnabar red with a short yellow pile.

Acrostichals in 6 rows, regular. No prescutellars. Anterior scutellars divergent. Thorax tannish yellow, shining, pleurae lighter. Anterior sternopleural ¼ to ⅓ posterior and much thinner. Legs pale yellow; apical bristles on first and second tibiae, preapicals on all three.

Abdomen yellow with diffuse brown marginal bands not interrupted in the middle and fading out laterally.

Wings clear. Two prominent bristles at apex of first costal section; third costal section with heavy bristles on its basal ⅖. Costal index 1.94 ± 0.02; 4th vein index 1.90 ± 0.02; 5x index 1.93 ± 0.02.

Length of body 1.79 ± 0.01 mm. (♂♂), 1.98 ± 0.01 mm. (♀♀).

Two anterior and two posterior Malpighian tubes, ends free. Testes yellow with about 5 coils, the distal portion containing spermatogonia much thinner than the middle. Spermathecae spherical, moderately chitinized, with a terminal indentation. Ventral receptacle a long tube forming a flat spiral bent into a W-shaped plate resting on the vagina.

Eggs—Two filaments expanded and flattened distally, about as long as the egg itself. Puparia—brownish yellow, horn very short, anterior spiracle with 9 short branches.

Chromosomes—metaphase plates show two pairs of V's, one of which is shorter than the other, and a pair of rods.

Distribution—Teffe, state of Amazonas, Brazil, collected in October 1943 by Th. Dobzhansky. The type now in the American Museum Natural History, New York.

DISCUSSION

D. equinoxialis and *D. willistoni* differ in the average body size; they differ also in the time of onset of mating after the hatching from the pupae. The differences are, however, so small that single individuals of the two species cannot be distinguished by inspection not only in museum material but even in living flies.

The geographic relationships of the two species are not clear. It is certain that *D. willistoni* is widely distributed in the American tropics, but *D. equinoxialis* may or may not be endemic to the Upper Amazon region where it was found. Since the two species were not distinguished in the field, only six living strains were established and brought to New York from many times that number of the flies collected. *D. willistoni*-like flies were found in several localities around Teffe, in the jungle as well as in gardens, but it is not clear whether all of them were *D. equinoxialis* or some of them were *D. willistoni.*

It is, therefore, uncertain whether *D. equinoxialis* and *D. willistoni* are wholly allopatric or sympatric in a part of their distribution ranges. Notwithstanding this uncertainty, there can be no reasonable doubt that these forms are full-fledged species rather than races of the same species. Indeed, the reproductive isolation between them is complete. The females and males discriminate between conspecific and alien prospective mates almost without fail, as the experiments summarized in tables I and II show. It is unlikely that the sexual

isolation could be less efficient under natural conditions than it is observed to be in laboratory experiments. However that may be, interspecific matings can lead to no gene exchange whatever, since they result in no viable progeny.

D. equinoxialis and *D. willistoni* are an extreme case of a morphologically similar pair of species, since in them an absolute reproductive isolation is combined with a virtually complete lack of morphological differences. It is interesting to compare this case with similar ones known in the literature. The reproductive isolation between the partly sympatric *D. persimilis* and *D. pseudoobscura* appears to be complete in nature, but experimental analysis reveals that this completeness is attained only through cooperation of several isolating mechanisms none of which is wholly effective by itself (Dobzhansky and Epling '44). Morphologically these species are identical, although Reed, Williams, and Chadwick ('42) have been able to distinguish the strains at their disposal by the ratio of thorax volume divided by the product of wing area times the cubed wing length.[1] *D. americana, D. texana,* and *D. novamexicana* are very similar morphologically, though not completely identical. Since they are mostly, though again not completely, allopatric in distribution, the reproductive isolation is probably not very important in nature. Laboratory experiments show it to be incomplete (Patterson '42, Patterson, Stone, and Griffen '40, '42).

D. americana, D. texana, and *D. novamexicana* are clearly close to the borderline between race and species. Since the reproductive isolation between them seems sufficient to make gene divergence outrun gene exchange, they are properly classified as species. *D. persimilis* and *D. pseudoobscura* are unquestionably species, since gene exchange between them is made impossible by co-action of several isolating mechanisms each of which is imperfect if taken by itself. The isolation between *D. equinoxialis* and *D. willistoni* is complete. These successive stages of evolutionary divergence are, however, not accompanied by morphological change. Speciation in Drosophila proceeds mainly through evolving physiological complexes which are successful each in its environment; the morphology of these flies seems to have reached an adaptive peak suitable in most of these environments.

[1] This has been misquoted as though the discriminating value were not a ratio but a power of a single character—wing length.

Summary

The only securely established morphological difference between *Drosophila equinoxialis* Dobzhansky and *Drosophila willistoni* Sturtevant is that the former species is on the average smaller than the latter. The variations are, however, broadly overlapping, so that inspection of a single individual may be insufficient to determine to which species it belongs. The average age at first mating is higher in *D. equinoxialis* than in *D. willistoni.* Despite their external similarity, males of either species rarely mate with females of the other, even if no choice of mates is available. No viable offspring results from interspecific inseminations. The reproductive isolation between the two species is, thus, complete.

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