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IV. *Drosophila insularis*, a New Sibling Species of the Willistoni Group

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INTRODUCTION

For about two centuries, insect species names were given to single specimens, or to series of specimens, of dried remains of insect bodies, preserved on pins in museum drawers. The chief criterion of validity of such "species" was that the specimens be distinguishable without undue trouble, preferably with the aid of no more than a simple hand lens, from other pinned specimens. The necessity of such "alpha taxonomy" for a preliminary orientation in the otherwise baffling and bewildering diversity of insect forms can hardly be questioned. "Alpha taxonomy" continues to be necessary in little known groups and in little known faunae.

However, after the taxonomic foundation has been laid, the methods of the so-called "new systematics" are made use of. New systematics studies species not as series of specimens but as biological phenomena. The "alpha taxonomists" have already had the intuition of the existence in nature, among sexually reproducing and cross-fertilizing organisms, of very significant biological units. These are Mendelian populations, or groups of such populations, within which the gene exchange normally occurs, but between which it is impeded or prevented by reproductive isolating mechanisms. The remarkable fact is that these reproductively isolated groups of populations coincide, in most cases, with the species of taxonomists. They coincide in most and yet not in all cases; the reproductive isolation, which is the touchstone of biological species in sexual organisms, is strongly but not absolutely correlated with easy morphological recognizability, which is the touchstone of "good" species of "alpha taxonomy."

The chief interest of systematics of *Drosophila* lies in that most of the insects belonging to this genus are singularly amenable to methods of investigation which shed light on species as biological phenomena. Most *Drosophilae* can be easily and cheaply maintained in laboratory cultures. A variety of tests disclosing the presence or absence of certain forms of reproductive isolation can readily be imposed. As material for ecological studies in nature *Drosophila* proved rather intractable; nevertheless, some ecological data can also be secured. In *Drosophila* one is, then, able in most instances to evaluate the degree of correspondence between the species of "alpha taxonomy" and biological species.

It is perhaps gratifying that by and large this correspondence amounts to identity, as shown most convincingly particularly by Patterson and Stone (1952) in their admirable work on the evolution of *Drosophila*. And yet some noteworthy exceptions have been found (see Patterson and Stone, page 180). The exceptions concern mainly sibling species. Sibling species have been defined by Mayr (1942) as species "which are morphologically very similar and indistinguishable, but which possess specific biological characteristics and are reproductively isolated." It would be premature at present to decide whether sibling

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species are more frequent among *Drosophila* than among other insects, although taking the data at their face value this would appear to be so. The work of Sonneborn and his school has shown that sibling species (there dubbed "varieties") are much more numerous among certain infusorians than they are among *Drosophila*. However that may be, and despite some disingenuous suggestions that have been made that *Drosophila* systematics should be kept within the bounds of "alpha taxonomy," the study of sibling species is certainly important because of the light which it sheds on the nature of species in general. The group of sibling species related to *D. willistoni* is perhaps one of the most interesting among those known in the genus.

THE WILLISTONI SPECIES GROUP

The *willistoni* species group is a subdivision of the sub-genus *Sophophora* of the genus *Drosophila*. Patterson and Stone (1952, pages 51 and 52) list nine species, and three further species have since been added to the group. They are all native in the Neotropical faunal region, although *D. willistoni* reaches southern Florida, and *D. nebulosa* occurs in southern Texas and has been found as far north as Nebraska, doubtless an accidental introduction by man. According to Burla *et al.* (1949), the sibling species related to *D. willistoni* are four in number, namely *D. willistoni* Sturtevant, *D. paulistorum* Dobzhansky and Pavan, *D. equinoxialis* Dobzhansky, and *D. tropicalis* Burla and da Cunha. A fifth sibling, *Drosophila insularis* Dobzhansky may now be added.

The siblings are morphologically very close. Although Burla *et al.* (1949) have listed several minor differences, both external and internal, identification of single specimens, even living ones, used to be practically out of the question. The situation has changed thanks to Spassky's discovery of slight but constant differences in the external male genitalia (Spassky, an article in this volume). Male specimens may now be identified as to the species. As correctly pointed out by Spassky himself, this does not alter the status of the five species as siblings. The morphological differences between these species are still so small that no one would recognize them as distinct species if it were not for other relevant evidence. This evidence shows that the species are completely isolated reproductively. Burla *et al.* (1949) found that the four sibling species known to them fail to cross. Insemination is rarely observed even when females of one species are confined with males of another in the absence of conspecific males (except that *D. paulistorum* females of the strain used accepted males of the other species relatively easily, though much less so than their own males). However, regardless of the frequency of insemination by foreign males, no hybrid larvae or adults have ever appeared. It remains unknown whether fertilization of eggs by foreign spermatozoa occurs at all, and if so at what stage the embryos die out.

At least equally decisive as the above evidence is the evidence that no gene exchange between the sibling species takes place in nature. Some of the chromosomes of the sibling species (especially the third) are distinguishable in the preparations of salivary gland cells, and the populations of each species contain species-characteristic inversions. If effective introgression were taking place, these cytological markers would occasionally be exchanged between the species. Rather extensive studies of natural populations failed to disclose a single indi-

cation of anything of this sort (da Cunha and Dobzhansky 1954, and much unpublished evidence).

The geographic distribution of the sibling species is interesting. *D. willistoni* is distributed most widely from the Argentine Pampa to the West Indies, southern Florida, and north-central Mexico (San Luis Potosi), and from the eastern slope of the Andes in Peru and northern Argentina to the Atlantic. The distribution areas of the other siblings seemed to be included into each other, in the order *willistoni-paulistorum-tropicalis-equinoxialis* (Burla *et al.* 1949), but the study of the abundant material collected by the expeditions of the genetics group of the University of Texas in Central America and the West Indies discloses a somewhat different situation (Dobzhansky 1957). Namely, *D. paulistorum* is more at home in the south, while its nearest relative, *D. equinoxialis*, has a more northern distribution. The former species occurs from southern Brazil (Rio Grande do Sul) to Colombia, Panama, Costa Rica and Salvador; it is often the dominant species in superhumid rainforest regions of Brazil, but it seems to be entirely absent in the West Indies, except in Trinidad. *D. equinoxialis* is, on the contrary, rare in Brazil, but it is frequent or even dominant in Central America and on the islands of the Greater Antilles. The Lesser Antilles have only *D. willistoni* and *D. insularis* (see below). *D. tropicalis* extends from central Brazil (Goyaz) where it is rare, to Central America and the Greater Antilles, where it is common and locally even dominant. It is tempting to speculate that *D. paulistorum* and *D. equinoxialis*, and *D. willistoni* and *D. tropicalis* were at some time in the past respectively the southern and northern subspecies of their ancestral species. After having evolved complete reproductive isolation, the now full-fledged sibling species all became sympatric in a large territory, embracing the Amazon Valley, the northern part of the continent of South America, a part of Central America, and the Island of Trinidad. The newly discovered, the fifth, sibling species, *D. insularis*, may now be described.

DROSOPHILA INSULARIS DOBZHANSKY, *SPECIES NOVA*

Male and female: Arista with 10-11 branches, both numbers being about equally frequent. Front dusky yellow. Anterior orbital bristles shorter than the posterior, middle orbital one-third posterior. Two prominent oral bristles. Face yellow. Carina short and broad, not sulcate. Cheeks yellow, their greatest width about one-tenth greatest diameter of eye. Eyes bright red with a short brownish pile.

Acrostichals in 6 to 8 rows, often quite irregular. Thorax dusky yellow, darker on the average and in flies of equal age than in *Drosophila willistoni* Sturtevant; pleurae lighter than the thorax. Anterior and middle sternopleurals at most half as long as the posterior and much thinner. Legs greyish yellow. Abdomen yellow with diffuse dark brown bands expanded in the middle and fading out laterally. Wings clear, proportions like in *Drosophila willistoni* but slightly darker in color, especially in old individuals.

Length of body in laboratory-bred specimens: ♀ 2.9-3.2 (mean 3.03), ♂ 2.5-2.8 (mean 2.65) mm.; wings ♀ 2.1-2.3 (mean 2.16), ♂ 2.0-2.1 (mean 2.04) mm.

Reproductive organs of the adults, eggs, larvae, and pupae like those in *Dro-*

sophila willistoni, except for minute but constant differences in the external male genitalia which will be described in a separate publication by Mr. B. Spassky.

Remarks. Closely related to *Drosophila willistoni*, from which it differs by a slightly larger body size and a darker pigmentation. These differences, though fairly clear in flies grown in similar environments, are not reliable for identification of single specimens grown in diverse environments. However, the reproductive isolation between *Drosophila insularis* and *Drosophila willistoni* is, as shown below, complete.

Geographic Distribution. In the sample of about 80 females of *willistoni*-like species collected on the Island of St. Kitts by Dr. W. B. Heed in January of 1956 and sent to the laboratory in Columbia University, 77 females which yielded progenies belonged to *D. willistoni* and 3 to *D. insularis*. The sample from the Island of St. Lucia collected by Dr. Heed in the same month contained 105 fertile females of *D. willistoni* and 1 of *D. insularis*. The samples from Trinidad, Barbados, or the Greater Antilles, contained no *D. insularis*. This species is probably confined to the Lesser Antilles, but how widespread it is on the islands of this chain cannot be decided from the data now available.

Chromosomes. The metaphase group consists of two pairs of metacentric and one pair of acrocentric chromosomes. The "dots" are absent. This metaphasic configuration is invariant in all known species of the *willistoni* group, and, with one unconfirmed exception, in all examined species of the second characteristically Neotropical group of the subgenus *Sophophora*, namely the *saltans* species group. The giant chromosomes in the salivary gland cells show slight but characteristic differences in the different sibling species of the *willistoni* group (Burla *et al.* 1949). Most characteristic are the disc patterns in the distal portions of the third chromosomes (cf. Plate 1, page 304, in Burla *et al.*, *l.c.*). The disc pattern of this chromosome in *D. insularis* is most nearly akin to that in *D. tropicalis*, except for the absence of the distinct light "bulb" located proximally from the group of heavy doublets characteristic of the latter species. A further resemblance of *D. insularis* to *D. tropicalis* lies in the disc patterns of the right limbs of the X-chromosomes (XR), which have the characteristic "repeat" area in a more nearly subterminal position, while in *D. willistoni* it is in a submedian position.

SEXUAL ISOLATION

As mentioned above, Burla *et al.* (1949) found that the four sibling species of the *willistoni* group known to them showed strong sexual isolation from each other. We have, consequently, studied the situation in this respect of the fifth sibling, *D. insularis*. The usual "multiple choice" method was used in the experiments, a mixture of females of two species being confined with males of one of them.

Newly hatched females and males were divided in groups of three. Three females of one species, three females of another, and three males conspecific with one of the kinds of females were placed together in small containers ("creamers") and left undisturbed for 8-10 days at room temperature (about 22° C. with fluctuations of several degrees). To make the identification of the females of the sibling species easy, the tips of the wings were clipped either on the right or on

the left side. The dissection of the females and the examination of the contents of their sperm receptacles under a microscope yielded the data summarized in Table 1. The isolation index was completed according to Stalker (1942).

TABLE 1

Numbers of females tested and per cent found inseminated in various crosses of *D. insularis* with other sibling species

Males	Females	Homogamic		Heterogamic		Isolation Index
		n	%	n	%	
<i>insularis</i>	<i>insularis</i> + <i>paulistorum</i>	95	96.8	94	1.06	0.976
<i>insularis</i>	<i>insularis</i> + <i>equinoxialis</i>	166	95.1	169	6.47	0.873
<i>insularis</i>	<i>insularis</i> + <i>tropicalis</i>	86	96.5	82	4.57	0.903
<i>insularis</i>	<i>insularis</i> + <i>willistoni</i> (St. Kitts)	180	92.3	180	3.8	0.879
<i>insularis</i>	<i>insularis</i> + <i>willistoni</i> (Recife)	182	94.5	182	10.3	0.791
<i>paulistorum</i>	<i>paulistorum</i> + <i>insularis</i>	159	96.85	155	5.03	0.901
<i>equinoxialis</i>	<i>equinoxialis</i> + <i>insularis</i>	72	100	72	8.33	0.846
<i>tropicalis</i>	<i>tropicalis</i> + <i>insularis</i>	171	98.2	171	8.76	0.836
<i>willistoni</i>	<i>willistoni</i> (St. Kitts) + <i>insularis</i>	180	100	180	1.59	0.957
<i>willistoni</i>	<i>willistoni</i> (Recife) + <i>insularis</i>	180	97.2	180	13.3	0.759

It can be seen that the sexual isolation is quite strongly pronounced in all the crosses studied. And yet, cross-inseminations have been observed in every one of the ten combinations of heterospecific parents which have been tried. In the experiments of Burla *et al.* (1949) several of the crosses between the sibling species of the *willistoni* group showed no insemination at all, and only the crosses to *paulistorum* males have a considerable frequency of inseminations. There seems to be, therefore, a real difference between the behavior of *D. insularis* with respect to the other sibling species and that of these sibling species with respect to one another. There is one further fact that should be noted. The crosses *insularis* ♀ × *willistoni* ♂ and *willistoni* ♀ × *insularis* ♂ have been made using two different strains of the *willistoni* parent, namely a strain from the Island of St. Kitts and from Recife, Brazil. Now, *D. insularis* shares the islands of St. Kitts and St. Lucia only with *D. willistoni* and with no other species. The crosses *D. insularis* × *D. willistoni* from St. Kitts are, therefore, crosses between strains which are sympatric in origin. The Recife strain of *D. willistoni* comes, on the contrary, from a locality quite remote from the islands in which *D. insularis* is known to live. The isolation coefficients in the crosses which involve the Recife strain are ostensibly, though not quite significantly, lower than those in the corresponding crosses with *D. willistoni* from St. Kitts. The chi-square test gives $0.05 < p < 0.10$ in both crosses.

INTERSPECIFIC HYBRIDIZATION

Systematic experiments have been made crossing *D. insularis* to the other four sibling species. All the hybrids so far obtained came from the crosses in which *D. insularis* was used as the female parent. It would, nevertheless, be premature to conclude that the reciprocal crosses are wholly incapable of producing viable hybrids, since perhaps an insufficient number of attempts have been made, and since insemination of females of other species by *D. insularis* males does occur (see above). The technique of the experiments was to place

several (up to about a dozen) virgin females with approximately equal numbers of males of a foreign species in a culture bottle. After a lapse of about a week, inspection disclosed the presence or absence of hybrid larvae. If hybrids were absent, the parents were transferred to fresh cultures, to give them another chance to produce hybrids. Flies of *D. insularis* from every one of the four St. Kitts strains were used in these experiments indiscriminately.

The cross *insularis* ♀ × *tropicalis* ♂ (the strain from Palma, Goyaz, Brazil) yields hybrids most easily. Several hundred such hybrids have been examined from a number of different cultures. Hybrids of both sexes appear, the sex ratio being about 1:1. The external morphology of the hybrids is apparently normal; study of the copulatory organs of hybrid males by Mr. Spassky (in press) showed these to be approximately intermediate between those of the parental species. The internal reproductive organs are also normal, except for the gonads themselves which are grossly abnormal. The testes of the males are reduced to tiny rudiments, smaller in size than the testes of fully grown larvae. No histological examination has been made, except for observation under an intermediate magnification (about × 600) of a microscope of freshly dissected gonads flattened under a cover-slip. This examination shows nothing resembling normal spermatogenesis. The remains of the testes consist of fairly large undifferentiated cells. The ovaries of the hybrid females are composed of normal-looking egg strings and ovarioles, in which the oocytes and the nurse cells are clearly differentiated. In the ovaries of hybrid females which are about a week old one finds abnormal eggs, with a formed chorion and with two filaments which are shorter than those in the eggs of either parental species. Such females were kept with males of both parental species, but, as expected, no progenies resulted. The hybrid females, like the hybrid males, are wholly sterile.

The crosses *insularis* ♀ × *willistoni* ♂ (using strains of *D. willistoni* derived from flies collected on the Island of St. Kitts) succeed not quite as easily as those to *tropicalis* males. Nevertheless, hybrids of both sexes have been obtained and studied. Their characteristics are quite similar to those of the *insularis* ♀ × *tropicalis* ♂ hybrids described above. They are completely sterile. The crosses *insularis* ♀ × *equinoxialis* ♂ (using the strain from Teffe, Amazonas, Brazil) produce mostly no hybrids at all. However, one cross yielded several pupae which failed to hatch, and in another cross five hybrid females, and no males, were obtained. These hybrid females were kept for some time in a culture with males of both parental species, but proved to be sterile. The cross *insularis* ♀ × *paulistorum* ♂ (a brown-eyed mutant strain) is most refractory. Some larvae have, however, been noted in one of the cultures, but they failed to produce adult hybrids.

A most interesting peculiarity of the *insularis* ♀ × *tropicalis* ♂ and the *insularis* ♀ × *willistoni* ♂ hybrids, which as far as we are aware has no analogue in any other interspecific hybrids, concerns the behavior of the chromosomes in the salivary gland cells of the hybrid larvae. This matter will be described in more detail elsewhere (Dobzhansky, in press in "Chromosoma"). The essence of the story is as follows. The heterochromatic parts adjacent to the centromeres in all the chromosomes come together in the hybrid cells to form a compact chromocenter which resembles the chromocenter of normal cells. However, the

euchromatic parts of the chromosomes of the two species evince no attraction toward each other, and remain completely unpaired. The hybrid males, thus, have eight chromosomal strands radiating from the chromocenter. Two of these eight strands are the two arms of the X-chromosome. Female larvae must have 10 chromosomal strands, four of them being X-chromosomes, but no cell has been found in which all strands were identifiable. The lack of pairing of the euchromatic chromosome strands certainly cannot be due to lack of linear homology, since the chromosomes remain easily recognizable as homologues by their disc patterns. Another interesting fact is that the X-chromosomes in the male larvae were clearly greater in average diameter than are the autosomes in the same cells, or than the X-chromosomes or the autosomes in female larvae. Anybody with any experience in studying the chromosomes in the salivary gland cells of *Drosophila* larvae knows that the X-chromosome in male larvae (which is single) differs in a paler color, but not in average diameter, from the X-chromosome of female larvae (which is double). The observations on the *insularis* ♀ × *tropicalis* ♂ hybrid larvae prove that this behavior of the X-chromosome depends upon the sex of the cell and not upon the chromosome being paired or unpaired.

VARIATIONS IN THE GENE ARRANGEMENT IN *D. INSULARIS*

The sibling species of the *willistoni* group tend to be highly polymorphic with respect to the gene arrangements in their chromosomes. In fact, natural populations of *D. willistoni* show a diversity of chromosomal structures which is, as far as we know, greater than that recorded in any other species. It is interesting that chromosomal polymorphism exists even in *D. insularis*, the populations of which are isolated on islands.

The four strains of *D. insularis* from St. Kitts were out-crossed to each other and to the strain from St. Lucia. As expected, the crosses went easily and the hybrids were fertile. Three different inversions exist in the St. Kitts population. One of them includes the sub-terminal portion of the right limb of the second chromosome (II R). The homologue of the heavily-banded "repeat" located in this chromosome, corresponding to the section 70 in the II R of *D. willistoni* (see the map in Dobzhansky, 1950), is included in this inversion. The position of this landmark in the chromosome is more nearly subterminal in *D. insularis* than in *D. willistoni*. Another inversion has been found in the subbasal portion of the left limb of the second chromosome (II L). This inversion, like that in II R, is short, but it includes no conspicuous landmark, and its position in terms of the standard map in *D. willistoni* has not been determined. The third inversion has been found in the subterminal portion of the third chromosome (III). All three inversions exist within the St. Kitts population of *D. insularis*, and the only available strain from St. Lucia happens to be free from inversions.

The precise limits of the inversions in *D. insularis* in terms of the maps of the salivary gland chromosomes have as yet not been determined. A comparison of the inversions in the species with those recorded in the other four siblings has nevertheless been made, using the published descriptions and the unpublished notes. The inversions in *D. insularis* are not identical with any found in other

species. Thus far, no inversion has been encountered in more than one of the five siblings.

CONCLUSIONS AND SUMMARY

Drosophila insularis resembles morphologically the other four sibling species of the *willistoni* group. It shows some slight differences in the external appearance, a difference in the structure of the male copulatory organs (discovered by Mr. B. Spassky), and differences in the disc patterns of the chromosomes in the salivary gland cells. Nevertheless, the reproductive isolation between any two of the five siblings is complete. Of the isolating mechanisms so far discovered, sexual isolation is probably most important in natural populations. Nevertheless, this isolation is not absolute, and cross-insemination has been observed under experimental conditions, especially when *D. insularis* females were exposed to males of the other species. Viable hybrids are produced particularly in the cross *D. insularis* ♀ × *D. tropicalis* ♂. These hybrids are, however, completely sterile in both sexes.

D. insularis is the only one of the five sibling species which is capable of producing some hybrids with every one of the remaining four siblings. These latter have never been observed to produce any progenies in the intercrosses with each other. The hybrids are, however, completely sterile, thus making *D. insularis* a reproductively absolutely isolated species. Sexual isolation has been tested between *D. insularis* and the other species with the aid of the multiple choice method. A pronounced isolation has been observed in every cross, though perhaps less strong on the average than the sexual isolation between the four sibling species other than *D. insularis*, as studied by Burla *et al.* (1949). It is tempting to correlate the fact that the isolating mechanisms between these latter species are more nearly perfect than those between them and *D. insularis* with the geographic distribution of these species. The four siblings other than *D. insularis* have their geographic distributions widely overlapping, so that they are sympatric in extensive territories in South and Central America. *D. insularis* is, on the other hand, endemic on some of the islands of the Lesser Antilles, and the only other species which occurs there is *D. willistoni*. Another interesting fact in this connection is that this sexual isolation between *D. willistoni* and *D. insularis* is at least ostensibly stronger if the strains tested are sympatric in origin than when they are allopatric in origin. All these facts are consistent with the hypothesis that reproductive isolating mechanisms between populations of sympatric species may be perfected owing to pressure of natural selection.

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