INCIPIENT REPRODUCTIVE ISOLATION BETWEEN TWO SUBSPECIES OF DROSOPHILA PALLIDIPENNIS

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INTRODUCTION

THE process of transformation of races into species is in general slow. The only approach to its study is through examination of its successive stages represented on our time level in different species and species groups. Nor is the process uniform in all organisms. Even though the essential feature of speciation in sexual cross-fertilizing forms is the development of reproductive isolation between the diverging races, the isolation is attained by different means in different cases. This necessitates careful study of diverse groups of organisms, as well as of different related species with contrasting reproductive biologies, modes of life, and distributional relationships.

For many years it appeared that species of Drosophila, though they offer some of the best material for investigation of many genetic problems, are not favorable for studies on speciation; it looked as though the intraspecific variation among natural populations is very small, while the reproductive isolation between species of this genus is almost always complete. In other words, the critical stages of speciation, the intermediates between races and species, seemed to be absent, as if species formation has come to a standstill. Facts brought to light during the last decade have, however, shown that the above impressions were erroneous, based on a failure to realize that the speciation in Drosophila is frequently accompanied by very little differentiation in the visible characters. Undoubtedly separate species of this genus may be morphologically similar and even identical, while morphologically distinguishable subspecies are, compared to other organisms, rare. It is now clear that the genus Drosophila, contrary to the old idea, is quite rich in "borderline cases" between race and species. Owing to the inherent advantages of many Drosophila species as laboratory materials, they offer excellent opportunities for studies on the genetics of speciation.

In the present article we wish to report on two subspecies of *D. pallidipennis* which have developed slight morphological differences and partial hybrid sterility, without either differentiation of the gene arrangements in their chromosomes or sexual isolation. We are obligated to MRS. N. P. SIVERTZEV-DOBZHANSKY for her help in making the wing measurements, and to MISS IRENE MARKREICH for technical assistance.

MORPHOLOGICAL DIFFERENCES

Our material consisted of a strain of *D. pallidipennis pallidipennis* from Iporanga, State of São Paulo, Brazil (collected by DR. C. PAVAN), and a strain of *D. pallidipennis centralis* from Jalapa, State of Vera Cruz, Mexico (collected by G. B. MAINLAND). After the work was almost completed we obtained another

strain of *D. pallidipennis pallidipennis* collected in the city of São Paulo by DR. C. PAVAN.

The published descriptions of the two subspecies (DOBZHANSKY and PAVAN 1943; PATTERSON and MAINLAND 1944) indicate that they are very close in external appearance, as well as in anatomical and cytological features. To obtain material for detailed comparison, flies of the Iporanga *pallidipennis* and the Jalapa *centralis* strians were transferred daily to fresh culture bottles, the number of parents being adjusted to prevent overpopulation. The cultures developed in a room at 20°–22°C. When the flies hatched, they were placed to harden for a day or two in fresh bottles with food, whereupon the body length was measured in 100 freshly etherized females and as many males of each of

TABLE 1

Body and wing lengths (in mm) and the wing indices of D. pallidipennis pallidipennis and D. pallidipennis centralis.

CHARACTER	SEX	PALLIDIPENNIS		CENTRALIS	
		M±m	σ	M±m	σ
Body length	♀	4.620±0.020	0.199	4.227±0.017	0.172
"	♂	4.245±0.014	0.141	3.982±0.014	0.141
Wing length	♀	3.680±0.017	0.116	3.252±0.017	0.120
"	♂	3.386±0.017	0.121	3.220±0.017	0.122
Wing length/width index	♀	1.970±0.009	0.061	2.103±0.009	0.065
"	♂	1.936±0.009	0.064	2.059±0.010	0.072
Costal index	♀	5.132±0.050	0.347	5.338±0.045	0.311
"	♂	5.108±0.049	0.347	5.242±0.050	0.353
4th vein index	♀	1.160±0.008	0.054	1.159±0.009	0.060
"	♂	1.163±0.010	0.070	1.145±0.012	0.083
5×index	♀	0.997±0.011	0.075	1.101±0.016	0.111
"	♂	1.035±0.012	0.084	1.195±0.016	0.112

the two subspecies. Then the flies were preserved in alcohol, and served later to make the wing measurements of 50 females and 50 males of each subspecies. In making the measurements the conventions indicated in DOBZHANSKY and PAVAN 1943) were observed. In addition to the usual measurements, "maximum wing width" was taken in order to compute the wing length: wing width index; "maximum wing width" is not generally a satisfactory measurement, since it must be taken between two not very well fixed points on the wing margins; it was taken in this work because an inspection of the wings of the two subspecies suggested that they differ in the length: width ratio. A summary of the results of the measurements is given in table 1.

The data in table 1 show that *pallidipennis* is a larger fly, with an absolutely longer but relatively narrower wing, a lower costal index, and a lower 5x index than *centralis*. But none of the distinctions are sharp, and the variation distributions overlap in all cases. The 4th vein indices do not differ significantly.

If *pallidipennis* and *centralis* are raised under identical conditions, it is possible, with practice, to classify mixed lots of etherized living flies with only a few mistakes. Attempts to classify mixed lots of flies raised in mass cultures

without special efforts to make the environments uniform were less successful, although still more than half of the flies were classified correctly. No attempt to classify dried flies was made. The principal characters used in the classification are the larger body size and the more rounded (instead of parallel-sided) wing shape in *pallidipennis*. It is also possible that the eye color of young flies is a little brighter, the eyes relatively larger and the cheeks relatively narrower in *centralis*, and the mesonotum darker and suffused with a more silky sheen in *pallidipennis*. Since we studied only a single strain of each subspecies, the objection may be raised that the distinctions cited are characteristic of these strains only and not of the subspecific populations as a whole. All we can say is that an inspection of the São Paulo *pallidipennis* strain showed

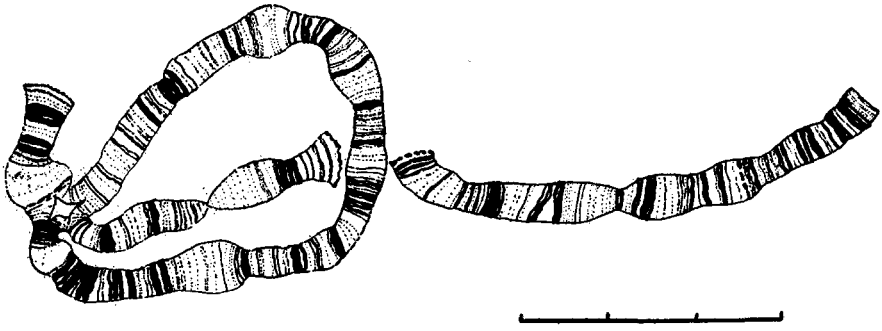

FIGURE 1.—Chromosome D in a salivary gland cell of a *D. pallidipennis pallidipennis*×*D. pallidipennis centralis* hybrid larva. The scale on the right represents 30 micra.

that it resembles the Iporanga *pallidipennis* rather than the Mexican *centralis* and that differences such as here described are seldom found between strains derived from the same population of a species of Drosophila.

CHROMOSOMES

The metaphase chromosomes of the two subspecies are similar. Salivary gland preparations were made of the F_1 hybrid larvae. Their examination disclosed that the pairing of the chromosomes in these hybrids is just as intimate as it is within either subspecies. Neither numerous unpaired sections nor the general lack of tautness, so characteristic of the salivary gland chromosomes in hybrids of species of Drosophila, were found. The only difference between the gene arrangement of *pallidipennis* and *centralis* is a fairly long inversion near the base of chromosome D found in all hybrid larvae. This inversion is shown in figure 1 (*cf.* fig. 15 in DOBZHANSKY 1944). A search for possible small rearrangements gave negative results.

Even if the above inversion should be found characteristic of the whole subspecies, and not simply of the strains at our disposal, the amount of chromosomal differentiation is still very low. Individuals heterozygous for several inversions are frequent in populations of many species of Drosophila; strains of the same species coming from regions as remote as Mexico and southern Brazil would, in general, be expected to differ in more than a single inversion.

TABLE 2

Crossability of D. pallidipennis pallidipennis (abbreviated as P) and D. pallidipennis centralis (abbreviated as C); the female parent is shown first.

	MATING	PERCENTAGE FERTILE		MATING	PERCENTAGE FERTILE
Controls	P×P	58		(PC)P)P×P	27
				(PC)C)P×P	26
	C×C	35		(CP)P)P×P	33
				(CP)C)P×P	30
P_1 crosses	P×C	76		(PC)P)P×C	38
				(PC)C)P×C	50
	C×P	35		(CP)P)P×C	34
				(CP)C)P×C	65
First backcrosses	PC×P	37		(PC)P)C×P	31
	PC×C	31		(PC)C)C×P	20
	CP×P	22		(CP)P)C×P	22
	CP×C	22		(CP)C)P×P	24
	P×PC	Sterile		(PC)P)C×C	20
	P×CP	Sterile		(PC)C)C×C	58
	C×PC	Sterile		(CP)P)C×C	18
	C×CP	Sterile		(CP)C)C×C	36
Second backcrosses	(PC)P×P	22	Third backcrosses	P×(PC)P)P	2
	(PC)C×P	25		P×(PC)C)P	Sterile
	(CP)P×P	21		P×(CP)P)P	5
	(CP)C×P	37		P×(CP)C)P	Sterile
	(PC)P×C	44		C×(PC)P)P	2
	(PC)C×C	35		C×(PC)C)P	1
	(CP)P×C	51		C×(CP)P)P	3
	(CP)C×C	46		C×(CP)C)P	Sterile
	P×(PC)P	Sterile		P×(PC)P)C	8
	P×(PC)C	5		P×(PC)C)C	6
	P×(CP)P	Sterile		P×(CP)P)C	Sterile
	P×(CP)C	1		P×(CP)C)C	10
	C×(PC)P	Sterile		C×(PC)P)C	Sterile
	C×(PC)C	Sterile		C×(PC)C)C	6
	C×(CP)P	Sterile		C×(CP)P)C	Sterile
	C×(CP)C	2		C×(CP)C)C	10

CROSSABILITY

Hybridization of *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* (denoted as P and C respectively in tables 2 and 3) succeeds without difficulty. The following technique has been evolved at the Texas laboratory to obtain quantitative data on the crossability and hybrid sterility in Drosophila crosses. Virgin females and males of the strains to be tested are selected and aged for three to five days in isolation; 132 pair matings are then made and placed in

$3\frac{3}{4} \times 1$ inch vials with food, one pair per vial. The vials are inspected on the fifth day after the mating, and any vials in which either of the prospective parents has died are discarded. On the twelfth day the vials are inspected again to record those in which offspring have and have not been produced. The number of vials among the first 100 examined which contain offspring is referred to as "percentage fertile" in table 2. It can be seen that 58 per cent of the vials contain offspring when *pallidipennis* females are crossed to *pallidipennis* males; when the same females are crossed to *centralis* males the percentage rises to 76 (the difference is probably not significant). With *centralis* females, 35 per cent of the matings produce offspring with either kind of the males.

The fecundity of the inter-racial crosses is lower than that of intra-racial matings. Fecundity is expressed as the average number of adult offspring produced by a single pair of parents under the conditions of our experiments (table 3). Approximately 35 offspring are produced by a pair of either *pallidipennis* or *centralis*. With *centralis* females crossed to *pallidipennis* males, this number falls to 16, and in the reciprocal cross it falls to only 8.

TABLE 3

Fecundity of crosses of D. pallidipennis pallidipennis and D. pallidipennis centralis (the female is shown first).

MATING	FECUNDITY
P×P	34.5
C×C	35.0
P×C	8.0
C×P	16.0
P×(PC)P)C	2.9
P×(CP)C)C	6.9
C×(PC)C)C	4.7
C×(CP)C)C	5.6

The causes of the reduction of fecundity in the inter-racial crosses are obscure. The adult F_1 hybrid flies show no indication of constitutional weakness compared to the representatives of the parental races. It is, therefore, rather improbable that a greater proportion of the hybrid than of the pure larvae succumb before reaching maturity. More likely, the lowered fecundity reflects either a lowered vitality of the sperm of one subspecies in the sperm receptacles of the other (as demonstrated in crosses of species of the *virilis* group by PATTERSON, STONE, and GRIFFEN 1942) or the delivery of fewer spermatozoa in inter-racial compared to intra-racial matings. This last possibility was suggested quite independently from the fecundity data by direct microscopic observations on the amount of sperm in the sperm receptacles of the females inseminated by males of their own or of the foreign subspecies.[1]

[1] The number of fertile eggs deposited by a female is, of course, only a fraction of the number of the spermatozoa transferred during copulation. The insemination mechanism in Drosophila is rather wasteful of spermatozoa (KAUFMANN and DEMEREC 1942). Although we did not attempt to count the spermatozoa, examination under a microscope suggested that the amounts of sperm in the receptacles vary quite considerably from female to female and that females inseminated by foreign males have fewer spermatozoa.

Observations on the amount of sperm in the receptacles were made in the course of experiments designed to test the possibility that an incipient sexual isolation may exist between the subspecies. Ten freshly hatched females of each of the two subspecies were confined with ten males of one of them for four days in $3\frac{1}{4}\times 1$ inch vials. The females were then dissected and their sperm receptacles examined for sperm under a microscope (for details of this technique see STALKER 1942, DOBZHANSKY and MAYR 1944). The results are summarized in table 4.

TABLE 4

Numbers of the females dissected and percentage of them containing sperm in experiments in which males of D. pallidipennis pallidipennis or D. pallidipennis centralis were confined with females of both subspecies.

MALES	*pallidipennis* ♀♀		*centralis* ♀♀	
	NUMBER DISSECTED	PERCENTAGE INSEMINATED	NUMBER DISSECTED	PERCENTAGE INSEMINATED
pallidipennis	106	55.7	106	33.0
centralis	111	42.3	109	42.2

Table 4 shows that equal proportions of females of the two subspecies are inseminated by *centralis* males; with *pallidipennis* males, a slightly but significantly higher proportion of *pallidipennis* than of *centralis* females are inseminated (the χ^2 is equal to 6.12, which, for one degree of freedom, is expected to occur by chance about once in 100 trials). Sexual isolation between the subspecies is, therefore, both weak and confined to only one of the two possible reciprocal crosses.

HYBRID STERILITY

Crosses of *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* produce hybrids of both sexes, which appear to be morphologically normal and at least equal to the parents in vigor. The F_1 hybrid males however, are, completely sterile. This has been established not only in pair matings (table 2) but also in mass cultures involving, in the aggregate, several thousand hybrid males. On the other hand, the F_1 hybrid females are fertile when backcrossed to males of either parental form. The percentage fertility tends to be lower in the F_1 hybrids than in the controls or in the P_1 crosses.

Extensive tests of the fertility of the hybrids obtained in the backcrosses have been made with the aid of the technique described above under the "Crossability" heading. Nearly 6,000 pair matings have been made for this purpose. The results are summarized in table 2. In this table the pedigree of each type of hybrid is indicated as follows. The original cross is given between parentheses, the females being always shown first. Thus (PC) is a hybrid from the cross *pallidipennis* ♀ × *centralis* ♂ and (CP) symbolizes the reciprocal cross. The male parent of the first backcross is shown next; thus, (PC)P is the offspring of an F_1 hybrid female crossed to *pallidipennis* male. The male

parent used in the second backcross is shown to the right of another parenthesis—(PC)P)P or (PC)P)C.

Females obtained in the offspring of the first backcross are fertile when backcrossed to males of either subspecies (see "Second backcrosses" in table 2); the same is true for the females obtained in the offspring of the second backcross (see "Third backcrosses" in table 2). The percentage fertility in these females is, on the average, about as high as it is in the F_1 females (see "First backcrosses" in table 2). However, the degree of fertility of the backcross females varies considerably—from 18 per cent in (CP)P)C to 65 percent in (CP)C)P. A part of this variation is doubtless due to chance, but there is a certain amount of evidence that some of the hybrid combinations are not as fertile as others, since the repetition of the crosses usually gives essentially the same results as did the original tests. It looks as though the backcross females tend to be on the average more fertile when crossed to *centralis* than when crossed to *pallidipennis* males, but this may or may not be significant.

In contrast to the complete sterility of the F_1 hybrid males, some of the males obtained in the backcrosses are fertile, although the percentage fertility is 10 or lower (table 2). It is significant that in the offspring of the second backcrosses the males derived from two backcrosses to the same subspecies are more likely to be fertile than those derived from backcrosses to different subspecies. Thus, the (CP)C)C, (CP)P)P, (PC)C)C, and (PC)P)P males are frequently fertile, while (CP)C)P, (CP)P)C, (PC)C)P, and (PC)P)C males are mostly sterile. In other words, males which carry half of the chromosomes of one subspecies and half of the chromosomes of the other are sterile, while the greater the preponderance of the chromosomes of one of the parental forms the greater the chance that a male may be fertile. This fact is compatible with the hypothesis that the sterility of the hybrids is due to interaction of several, possibly of many, genetic factors contributed by the two subspecies. The sterility of the *pallidipennis*×*centralis* hybrid males seems, therefore, to be comparable with that of the male hybrids between *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY 1936).

The fecundity of the fertile males obtained in the offspring of the backcrosses is very low—such a male mated to a single female of either subspecies produces less than ten offspring on the average (table 3).

SPERMATOGENESIS IN THE STERILE MALES

Species of Drosophila having spiral testes are less favorable as material for studies on spermatogenesis than species having ellipsoid testes. *D. pallidipennis* has spiral testes which contain relatively few spermatogonia, spermatocytes, and division figures even in freshly hatched males. No detailed study of spermatogenesis has, therefore, been made either in the pure forms or in the hybrids. Nevertheless, some aceto-orcein smear preparations of the testes have revealed the essentials of the cytological situation with enough clarity.

Both subspecies of *D. pallidipennis* have five pairs of chromosomes, one of these pairs being dot-like autosomes too small to be visible in the meiotic cells. About a dozen clear diakinesis and first metaphase configurations were ob-

served in the spermatocytes of the hybrid males, and all of them showed four bivalents, just as the corresponding cells in the normal males. The first meiotic division is, nevertheless, abortive. All the chromosomes become included into a single restitution nucleus which shows the diploid number of dyads, the equational halves of which flair widely apart; no cell division takes place. The second meiotic division is apparently also abortive; at any rate, one can see many nuclei with approximately the tetraploid number of chromosomes now reduced in size. Groups of very abnormal spermatids finally degenerate to form granular masses which fill the proximal end of the testis in the hybrid males.

Normal meiotic pairing of the chromosomes followed by abortive divisions and degeneration of the spermatids may seem a surprising sequence of events. Yet, this sequence has been observed also in the hybrids between "weak" strains of *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY 1934). The sterility of these latter hybrids is known to be genic rather than chromosomal; the degenerative phenomena in the spermatocytes and spermatids are not causally related to any lack of correspondence between the gene arrangements in the chromosomes of the parental forms. This is certainly true also in the *pallidipennis*×*centralis* hybrids, since in this case the parents differ in only a single inverted section (see above), which assuredly can not be responsible *per se* for the failure of spermatogenesis.

DISCUSSION

Drosophila pallidipennis pallidipennis and *D. pallidipennis centralis* are the products of a process of evolutionary divergence which seems to have reached the stage of speciation in the strict meaning of that word: these forms are on the "borderline" between race and species. They are distinguishable morphologically, at least in living flies raised under standardized conditions; yet, they show no more than a trace of the sexual isolation so characteristic for full species of Drosophila. The gene arrangements in their chromosomes differ in but a single inversion, yet the F_1 hybrid males are completely sterile and the males in the offspring of the backcrosses mostly sterile. Morphological differentiation, divergence of the gene arrangements, and the development of sexual isolation and hybrid sterility are the usual components of speciation in Drosophila. The example of *D. pallidipennis* shows that these components need not progress simultaneously.

Some closely related, though unquestionably distinct, species of Drosophila, such as *D. pseudoobscura* and *D. persimilis*, are scarcely if at all distinguishable morphologically; *pallidipennis* and *centralis* on the other hand are so distinguishable. The question may then be asked: why should the latter forms be regarded subspecies and not full species? Although it must be admitted that we are dealing here with a "borderline case," and that the information at hand is far from complete, the course adopted seems justified if systematic categories are to express the biological value of the populations concerned and not merely the degree of morphological distinctiveness. The distribution areas of *D. pseudoobscura* and *D. persimilis* broadly overlap, and yet no trace of gene exchange

has been discovered in the region in which these species are sympatric. This proves that reproductive isolation between these forms is strong enough to make their genotypes closed systems in nature. The distribution areas of *pallidipennis* and *centralis* are, unfortunately, little known. The strains at our disposal came from southern Brazil and from Mexico respectively, and no related forms have been recorded in the vast intervening territory, except perhaps DUDA's (1925) *D. hyalipennis* from Peru which appears, judging from the description, to be a fairly distinct species. Our estimate of the degree of reproductive isolation between *pallidipennis* and *centralis* rests on inference.

Laboratory experiments indicate little if any sexual isolation between *pallidipennis* and *centralis*, and no trace of inviability of their hybrids. Therefore, it is fair to assume that if populations of these forms were living side by side, numerous hybrids between them would be produced. To be sure, the propagation of these hybrids would be handicapped by the sterility of the males and the lowered fecundity of the females. However, the female hybrids are fertile enough so that the backcrosses would constitute a channel for extensive gene exchange. The lack of inversions to prevent crossing over in four out of the five large chromosomes means that gene recombinations will be formed freely. In the absence of evidence to the contrary, we believe that *pallidipennis* and *centralis* have not become reproductively isolated to an extent sufficient to maintain their genetic independence without geographical isolation. In other words, they are subspecies rather than species.

Even though *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* are regarded as subspecies, the great similarity of their gene arrangements makes them the most extreme case known in Drosophila of a failure of chromosomal differentiation to accompany the genetic divergence which apparently leads to speciation. This raises the general problem of the role played by chromosomal differentiation in evolution.

SUMMARY

Drosophila pallidipennis pallidipennis and *D. pallidipennis centralis* are known to occur in southern Brazil and in Mexico respectively. The strains examined show slight morphological differences sufficient to distinguish the subspecies in the living material raised under standardized conditions but probably not in dried specimens raised in different environments.

The gene arrangements in the chromosomes of the two subspecies are identical, except for an inversion in one of the autosomes. The pairing of the homologous chromosomes in the salivary gland cells of the hybrids is as intimate as it is in the parental forms. The subspecies cross readily and produce fully viable hybrids. No sexual isolation is apparent. The F_1 hybrid males are completely sterile, but the females are fertile. Females obtained in the offspring of the backcrosses are fertile. Some of the males obtained in the offspring of the first backcross are fertile, and the proportion of fertile males increases in the offspring of the second backcross. Taken as a whole, the data suggest that the sterility of the hybrids is caused by interaction of several, perhaps of many,

genes contributed by the parental forms. Gross disturbances are apparent in spermatogenesis of the sterile hybrid males, although the meiotic chromosome pairing seems to be normal.

The combination of a slight but perceptible morphological differentiation with the near-identity of the gene arrangement, and of the lack of sexual isolation with hybrid sterility suggests that the different components of the process of speciation characteristic for the genus Drosophila are largely independent.

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