MATING ACTIVITY OF HOMO- AND HETEROKARYOTYPES IN *DROSOPHILA PAVANI*¹

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Received October 9, 1963

MANY species of *Drosophila* exhibit chromosomal polymorphism due to inversions of chromosome sections. Species differ in the number, location, size, and frequency of the inversions. The differences can sometimes be correlated with the distribution, abundance, and other ecological characteristics of the populations, as well as with the physiological properties of the carriers of the inversions (review in DOBZHANSKY 1951; DA CUNHA 1960; SPIESS 1962). Two kinds of polymorphic species may be distinguished: (1) Species or populations in which the polymorphism is "flexible", i.e., the frequencies of the arrangements are modified by environmental changes, both in nature and under laboratory conditions. Some populations of *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY 1956), *D. robusta* (CARSON 1958), *D. subobscura* (SPERLICH 1961) and *D. flavopilosa* (BRNCIC 1962) belong to this group. (2) Species or populations with more rigid polymorphisms, in which there is no good evidence for geographical, seasonal, or altitudinal fluctuations of the frequency of polymorphic inversions. Rigid polymorphisms seem to be present in some populations of *D. robusta* (CARSON 1958), *D. subobscura* (KUNZE-MÜHL, MULLER and SPERLICH 1958), *D. willistoni* (DOBZHANSKY 1962) and *D. pavani* (BRNCIC 1957). Within a single Mendelian population, flexible and rigid polymorphism may both be present, but for different chromosomal arrangements (DOBZHANSKY 1962).

The reasons why a chromosomal polymorphism in *Drosophila* may be more rigid or more flexible are not well known; this may depend on the selective values of the homozygotes (homokaryotypes) and heterozygotes (heterokaryotypes) within each population. A selection which would favor homo- or heterokaryotypes according to the environment, will give a flexible polymorphism. On the other hand, if selection favors the heterokaryotypes in most of the environments which the species normally encounters, a more stable or rigid polymorphism will be established ("heteroselection" according to CARSON 1959). That some heterokaryotypes in *Drosophila* populations are strongly homeostatic, and superior in Darwinian fitness to homokaryotypes, has been demonstrated in some instances (see DOBZHANSKY 1951; SPIESS 1962).

The neotropical species, *Drosophila pavani*, (BRNCIC 1957), which lives in

¹ This work has been partially supported by grants from the School of Medicine, University of Chile, in a joint program with the Rockefeller Foundation.

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central Chile, and on the eastern slope of the Andes in Argentina, seems to be a good example of a species with a stable polymorphism. Its populations are polymorphic for complex gene arrangements in the second and fourth chromosomes (BRNCIC 1957), and in all the populations studied, the heterozygotes for these gene orders occur with frequencies close to 50 percent (BRNCIC 1957, 1959). In spite of the fact that some of the localities analyzed are relatively far from each other, that some of them are strongly isolated by geographic barriers, and have quite different environments, no appreciable geographical, seasonal or altitudinal fluctuations of the frequencies of these chromosomal variants have been observed. In stocks maintained for over ten years under the usual laboratory conditions, it has been observed that although the frequencies of heterokaryotypes have suffered some variations, they remain, in general, just as high as in the natural populations from which they came. Only in some laboratory stocks has there been a slight increase in the frequency of heterokaryotypes. Moreover, some unpublished experiments and observations of one of the authors (BRNCIC) have shown that the polymorphism in *D. pavani* remains stable when the stocks are subjected to environmental modifications of temperature and light.

A possible explanation of the rigidity of the polymorphism in *D. pavani* is that the chromosomal variants now found in the natural populations represent the final products of a long evolutionary process which has allowed only the persistence of the standard and the complex rearrangements (which differ from each other by many overlapping inversions), while all the intermediate links have been lost. After such a long and continuous selective pressure, which may have favored mainly the heterokaryotypes, it could be expected that these have become strongly heterotic in most of the environments encountered by the fly. Thus, the superior Darwinian fitness of the heterokaryotypes will be the main cause for the maintenance of the polymorphism in this species.

In searching for such heterotic properties of the heterokaryotypes in *D. pavani*, it was found that the heterozygotes for some gene arrangements had a greater longevity as compared to the corresponding homokaryotypes (BRNCIC and DEL SOLAR 1961). In the present paper the authors wish to discuss another physiological characteristic of the heterokaryotypes in this species, namely their superior mating ability, and its role in the maintenance of the polymorphism.

MATERIALS AND METHODS

In the experiments to be described, two strains of *D. pavani* were employed. These originated from flies collected in two biogeographic regions of Chile: Copiapó (Atacama) in the arid northern part of the country, and Bellavista (La Florida, Santiago) in the less arid central part. Both stocks were established in the following fashion: ten cultures, each with the offspring of ten females inseminated in nature which had been bred in individual vials. The offspring from the ten bottles were then mixed in each following generation, and the cultures were thereafter maintained in a large number of bottles. Thus, when the experiment began, each stock was derived presumably from 100 females inseminated in nature, and must have been strongly heterozygous.

When the present study was initiated, the stock from Bellavista had been in the laboratory for about ten generations and that from Copiapó for about eight generations. Both stocks were

polymorphic for the gene arrangements in their second and fourth chromosomes. In chromosome 2, besides the "Standard" gene order, there was the $A + B$ arrangement. In chromosome 4, besides "Standard" there existed in the right arm a rearrangement made up of three overlapping inversions (inversion IV-R; $A + B + C$), which are always found together, and in the left arm was present the (inversion IV-L; $A + B + C$) rearrangement, also made up of three overlapping inversions. For a description of these gene arrangements, see BRNCIC 1957. We shall be concerned only with the inversions in chromosome 4, the heterozygotes for which were present in a rather high frequency in both stocks. At the beginning of the experiments, in the Bellavista stock, the frequency of heterokaryotypes for the right arm of the fourth chromosome was 55 percent, and for the left arm, 48 percent. In the Copiapó stock these frequencies were 49 and 51 percent, respectively. The gene arrangement complexes in the right and left arms of the fourth chromosome were nonrandomly associated, as occurs in most of the laboratory stocks of *D. pavana* (BRNCIC 1961); therefore there was an excess of homozygotes and heterozygotes for both arms of the fourth chromosome. Both in the Copiapó and in the Bellavista stocks, 43 percent of the individuals were such double heterozygotes.

Experiments: Newly hatched males with no previous sexual experience from the stocks of Copiapó and Bellavista, were aged for ten days in 60 ml vials with the usual *Drosophila* medium in an incubator at 24°C. After this period, individual males were transferred without anesthesia to 8 ml empty vials together with a single ten day old virgin female from a stock homozygous for the Standard gene arrangements. At first, females from different homozygous stocks of *D. pavana* were used, but the extremely low reactivity of these females towards the males, low viability, and slow development, made the authors turn to homozygous females from a stock of the sibling species *D. gaucha* from Muitos Capoes (Brazil). Previous studies (KOREF-SANTIBAÑEZ and DEL SOLAR 1961; KOREF-SANTIBAÑEZ 1963) have shown that there is no sexual isolation between *D. pavana* and *D. gaucha* and that they easily produce F_1 hybrids (KOREF-SANTIBAÑEZ, CASANOVA and BRNCIC 1958). The following data are from the experiments performed with the females of *D. gaucha*.

Each pair was observed under a lamp at room temperature (22–23°C) over a period of 30 minutes, after which the flies were transferred to a culture vial with food medium enriched with live yeast and maintained in an incubator at 16°C until larvae appeared. The details of the courtship in *D. pavana* have been described by KOREF-SANTIBAÑEZ (1963). The cultures were divided into three groups according to the mating activity of the males: Group 1 included the pairs which copulated within the 30 minutes of observation; Group 2 included the pairs which showed courting activity but did not mate while under observation; Group 3 included the pairs in which no activity was observed during the 30 minute period.

The salivary gland chromosomes of eight larvae from each vial were prepared by means of the acetic-orcein rapid squash method, and examined under the microscope in order to determine whether the male progenitor was a structural homo- or heterozygote. The observation of eight larvae renders the possibility of misclassification small (1:128). The chromosomal constitution of about 100 males from each group in each stock was examined.

RESULTS

Table 1 shows that most of the males which mated under observation within 30 minutes produced progenies. This proportion decreases significantly in the cultures derived from pairs which did not mate within this period, and it is lowest in the group which showed no sexual activity at all. Some cultures failed to give offspring because of accidental death of the females, contamination of the cultures by bacteria or molds, laboratory accidents, etc. Since all these causes act uniformly in all the experimental groups, and since all the females belong to a rather homogeneous group, the differences observed depend on the sexual success of the males. Those males which did not mate within the first 30 minutes of contact with a female, usually fail to do so later on.

TABLE 1

Numbers of pairs of flies which copulated, courted or were inactive during the 30 minute observation periods, and the numbers and percentages of these pairs which produced progenies in further tests

Behavior	Copiapo stock			Bellavista stock		
	Observed pairs	Pairs producing progeny	Percent	Observed pairs	Pairs producing progeny	Percent
Copulated	126	119	94.4	133	100	75.2
Courted but did not copulate	195	118	60.5	298	100	33.5
Inactive pairs	248	137	55.2	417	120	28.7

Both in the Bellavista and in the Copiapó stocks there exist clear differences in the karyotypic composition of the males of the three groups (Table 2). As has been said previously, the gene arrangements in the fourth chromosome of *D. pavani* are nonrandomly associated. Nevertheless, in the stocks employed, a sufficient number of individuals was heterozygous for the gene arrangement in one of the arms, and homozygous for that in the other, to justify the analysis of the genetic constitution of each arm separately. In both stocks, it can be seen that among the males which mated or courted during the first 30 minutes of contact with the female, there is a significantly greater proportion of heterokaryotypes for the right and left arms of the fourth chromosome, as compared with the males which showed no sexual activity within this period. On the one hand, there are no appreciable differences between the groups in which mating or only courtship was observed during the period of observation. However, it is important to consider that the group which only courted and that of males which remained inactive are similar in their capacity for giving offspring. This paradoxical situation is obscure, and no satisfactory explanation can be given at present.

TABLE 2

Observed and expected numbers of males which were heterozygous for the gene arrangement in the fourth chromosome, among the groups which copulated, courted, or were inactive during the 30-minute observation periods

Strain and behavior	Number observed	Chromosome 4-R heterokaryotypes		Chromosome 4-L heterokaryotypes	
		Observed	Expected	Observed	Expected
Copiapó					
1. Copulated	100	65	53.6	53	48.3
2. Courted	100	63	53.6	57	48.3
3. Inactive	100	33	53.6	35	48.3
Chi-square		27.6		12.7	
		P > 0.0001		P > 0.002	
Bellavista					
1. Copulated	100	68	55.3	60	54.4
2. Courted	100	61	55.3	63	54.4
3. Inactive	120	48	66.4	51	65.2
Chi-square		20.9		12.6	
		P > 0.0001		P > 0.002	

DISCUSSION

Research on sexual behavior in *Drosophila* has shown that the carriers of different genotypes may differ in their mating ability. BASTOCK (1956), PETIT (1958), BÖSIGER (1960) and others found that in *D. melanogaster* some genotypes are more efficient than others in mating. RENDEL (1944) and MAYNARD SMITH (1956) have observed similar situations in *D. subobscura*. In *D. persimilis*, SPIESS and LANGER (1961), while searching for elements of fitness conferred by various homokaryotypes within a population, found significant differences in mating propensity.

The experiments on *D. pavani* show that males heterozygous for gene arrangements in their fourth chromosome are superior in mating ability to the corresponding homokaryotypes within the same population. They court and mate more rapidly. This has interesting evolutionary implications. The Darwinian fitness is a measure of the genetic contribution of the carriers of a genotype to the next generation. In cross-fertilizing organisms, the mating ability is an important component of fitness. Any selective pressure which modifies the courtship or mating efficiency, as SPURWAY (1955) and others have emphasized, may introduce a nonrandom element into the process of fertilization. If a greater mating ability is a property of a certain genetic constitution, and is correlated with characteristics that confer higher fitness, one may rightly suppose that it would contribute towards the maintenance of well balanced genotypes in the offspring.

In several species, namely *D. melanogaster* (BÖSIGER 1960; HOENIGSBERG and KOREF-SANTIBAÑEZ 1959) and *D. subobscura* (MAYNARD SMITH 1956), it has been observed that outbred lines exhibit greater and more efficient mating activity than inbred ones. This would be an expression of the heterotic properties of the heterozygotes in relation to homozygotes. Many other experiments have proven that heterokaryotypes are superior in viability, fertility, rate of development, longevity and other characters of importance in Darwinian fitness (reviewed in DOBZHANSKY 1951; DA CUNHA 1960; SPIESS 1962). The fact that in *D. pavani*, heterokaryotypes for complex inversions within the same Mendelian population court more actively, precociously, and have a greater mating success, may also be considered a manifestation of heterosis. That the heterokaryotypes in *D. pavani* are heterotic also with respect to other traits (such as longevity), has been demonstrated in previous work (BRNCIC and DEL SOLAR 1961).

Hypothetically, as was suggested in the introduction, the flexible chromosomal polymorphism which is exhibited by some populations of *D. pseudoobscura*, *D. persimilis*, *D. robusta* or *D. flavopilosa* (BRNCIC 1962) could indicate that both the homo- and heterokaryotypes have specific selective values according to the environments which they inhabit. In contrast, the more "rigid" polymorphism which is found in *D. pavani* and other species could be an expression of the stronger heterotic properties of the heterokaryotypes which manifest themselves under most environmental conditions in which the species is found. Therefore, if a greater mating activity, such as observed in *D. pavani*, represents one of the components of a superior fitness, it may be concluded that within a natural popu-

lation, sexual selection constitutes an important factor in the maintenance of genetic polymorphism.

The authors wish to acknowledge the valuable aid of Mrs. M. D. PELLICER and Mrs. A. CASANOVA. They also wish to express their gratitude to PROFESSOR TH. DOBZHANSKY of the Rockefeller Institute for his suggestions and criticisms of the manuscript.

SUMMARY

Drosophila pavani is a species with a stable polymorphism for chromosomal inversions. Heterokaryotypes are found with uniform frequencies in most populations studied, and also under different laboratory conditions. These heterokaryotypes are superior in Darwinian fitness to the homokaryotypes. This superiority is responsible for the maintenance of the chromosomal polymorphism in the populations. In searching for the physiological characteristics of the heterozygotes which could explain their superior fitness, the mating activity was studied in two very heterozygous Chilean populations, one from Copiapó, the other from Bellavista. Courtship of ten-day old males of each of these stocks, confronted individually with ten-day old virgin females from a stock of *D. gaucha* with a standard gene arrangement, was observed over a period of 30 minutes. The males were then classified into three groups according to their sexual activity: (1) those which mated within the period of observation; (2) those which only courted but did not copulate; (3) those which were sexually inactive during the observation period. The salivary gland chromosomes of eight larvae from the progeny of each pair were studied in order to determine the genetic constitution of the male parent. The frequency of heterokaryotypes was significantly higher among the males which mated and courted than in those which remained inactive. These results indicate that the greater mating activity is an expression of the heterotic properties of the heterokaryotypes. It may be an important factor in the maintenance of balanced polymorphism in the natural populations.

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