

A COMPARATIVE STUDY OF CHROMOSOMAL POLYMORPHISM IN CERTAIN SOUTH AMERICAN SPECIES OF *DROSOPHILA*

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I. INTRODUCTION

NATURAL populations of most species of *Drosophila* are variable with respect to the gene arrangement in the chromosomes of different individuals. In some species, a considerable proportion of flies found in nature are inversion heterozygotes. The degree of the variability of the chromosome structure varies however in geographic races of the same species, as well as in different species. To account for the different variability of different races and species, da Cunha, Burla and Dobzhansky (1950), and Dobzhansky, Burla and da Cunha (1950) have advanced the following working hypothesis. Since the chromosomal polymorphism in *Drosophila* populations is adaptive and balanced, the amount of this polymorphism present in a race or a species is likely to be a function of the number and variety of the ecological niches which the population has mastered. Other things being equal, species which exploit many different kinds of food, or which occur in many different biotopes, are likely to exhibit a greater degree of adaptive polymorphism than do species which are specialised to exploit only a limited variety of adaptive opportunities.

The data reported in the present paper have been collected in order to submit the above working hypothesis to further tests. We have chosen several species of *Drosophila* which are favourable for studies on the chromosomes in the larvæ salivary glands. The amounts of the chromosomal variability were then compared either in pairs of closely related species, or in different populations of the same species. The data so obtained can be evaluated against the background of the ecological characteristics of the respective races and species described by Dobzhansky and Pavan (1950), Pavan (unpublished), and Cordeiro (unpublished).

2. METHODS

Single females collected in nature were allowed to produce offspring in individual cultures with banana-agar nutrient medium. The salivary glands of mature larvæ produced in the cultures were stained with acetic orcein. The chromosomes of a single larva in the offspring of each wild progenitor were then examined.

3. *DROSOPHILA POLYMORPHA* AND *DROSOPHILA CARDINOIDES*

D. polymorpha Dobzhansky and Pavan and *D. cardinoides* Dobzhansky and Pavan are closely related species belonging to the *cardini* species group of the Subgenus *Drosophila*. They are sympatric in a large area, which extends, as far as known, from northern Argentina to Central America. The metaphase chromosome groups of both species are alike; they contain two pairs of metacentric autosomes (the second and the third), a pair of acrocentric X-chromosomes, and a pair of dot-like autosomes. The inversions found in these chromosomes are described by da Cunha and Salzano (in press).

TABLE 1

Frequencies (in per cent.) of various inversions found in heterozygous condition, and the mean numbers of heterozygous inversions per individual in *D. polymorpha*

Chromosome	Inversions	Vila Atlantica	Pirassununga	Mogí das Cruzes
II	{ none	71.6	98.8	98.7
	{ <i>Aa</i>	28.4	1.2	1.3
III	{ none	92.5	100	100
	{ <i>Aa</i>	7.5
	{ <i>Bb</i>	7.5
X	{ none	43.3	63.6	82.5
	{ <i>Aa</i>	43.3	36.4	17.5
	{ <i>Bb</i>	56.7	36.4	17.5
	{ <i>Cc</i>	43.3	...	17.5
Mean per individual	{ ♂ . . .	0.46 ± 0.08	0.01	0.01
	{ ♀ . . .	1.82 ± 0.18	1.10 ± 0.16	0.54 ± 0.14
Flies tested	{ ♂	67	83	93
	{ ♀	67	88	63

The chromosomal polymorphism in *D. polymorpha* is greater than that in *D. cardinoides*. In the former species, six different inversions have been found in 461 wild individuals collected in three localities, while in *D. cardinoides* three inversions were found in 141 flies, also from three localities. More important are the data on the frequencies of heterozygosis for inversions in the populations of the two species, summarised in tables 1 and 2.

Sufficient samples of *D. polymorpha* were available from three localities in the State of São Paulo, Brazil, namely Vila Atlantica, Pirassununga, and Mogí das Cruzes. The mean numbers of heterozygous inversions, per female, were 1.82, 1.10 and 0.54 in the three localities. The mean numbers in the males are lower, since no

inversion heterozygosis can be detected in the X-chromosome in the males.

Samples of *D. cardinoides* were studied from Vila Atlantica (State of São Paulo), from Cantareira (same State, about 50 km. from Mogí das Cruzes), and from Catuní (State of Bahia). In all these samples the inversions proved to be rare. The numbers of heterozygous inversions per individual were 0.06, 0 and 0.05 in the three localities respectively.

D. polymorpha is a species which is relatively more common, at least in southern Brazil, than *D. cardinoides*. At Pirassununga, the frequencies of *D. polymorpha* vary from 0.2 to 22 per cent. of the total catch of all species of *Drosophila*, depending upon season. *D. cardinoides* is much less frequent, varying from 0.03 to 0.5 per cent.

TABLE 2

Frequencies (in per cent.) of various inversions found in heterozygous condition, and the mean numbers of heterozygous inversions per individual in *D. cardinoides*

Chromosome	Inversions	Bragança	Cantareira	Vila Atlantica	Catuni
II	none	83	100	100	94
	<i>Aa</i>	17
	<i>Bb</i>	6
III	none	100	100	93	100
	<i>Aa</i>	7	...
Mean per individual . . .		0.16	0	0.06 ± 0.04	0.05 ± 0.03
Flies studied		6	50	46	55

of the total catch. At Mogí das Cruzes, *D. polymorpha* varies from 0.9 to 22 per cent., and *D. cardinoides* from 0.01 to 3.5 per cent. of all *Drosophila*. At Vila Atlantica, from 0.2 to 3 per cent. of the flies are *D. polymorpha* and 0.1 to 4.3 per cent. are *D. cardinoides*. It should be noted that the high proportions of *D. cardinoides* have been observed close to human habitations, and the proportions in undisturbed localities are always below 1 per cent. Similarly, at Cantareira and at Catuní, *D. cardinoides* has been collected only in orchards, near human habitations. *D. polymorpha* is a species which we have not collected under such conditions.

4. *DROSOPHILA GUARAMUNU* AND *DROSOPHILA GRISEOLINEATA*

D. guaramunu Dobzhansky and Pavan and *D. griseolineata* Duda are closely related to each other, and both of them belong to the *guarani* species group of the subgenus *Drosophila* (King, 1947). Their known distribution regions extend from northern Argentine to central

Brazil. In both species, the metaphase chromosome plates contain five pairs of rod-shaped and one pair of dot-like chromosomes. A total of 16 kinds of inversions have been found in the offspring of 896 wild individuals of *D. guaramunu* from seven different localities. A description of these inversions can be found in the paper of Brncic (in press). In *D. griseolineata*, 5 kinds of inversions have been encountered in the offspring of 612 wild flies from six localities. These inversions are to be described by Salzano (unpublished).

TABLE 3

Frequencies (in per cent.) of various inversions in heterozygous condition, and the mean numbers of heterozygous inversion per individual of *D. guaramunu*

Chromosome	Inversions	Chapada	Ponta Grossa	Itapoan	Feliz	Bragança	Mogí das Cruzes	Pirassununga
II	None	60.8	56.4	60.0	48.5	45.0	50.8	47.5
	Aa	39.2	43.6	40.0	51.5	55.0	49.2	52.5
III	None	43.4	41.8	50.0	51.5	45.0	49.2	47.9
	Aa or Bb	56.6	47.1	50.0	41.0	50.0	43.4	47.9
	Aa Bb	...	10.9	...	7.5	5.0	7.4	4.1
IV	None	32.6	74.5	30.0	30.3	30.0	49.2	40.1
	Aa	19.5	3.6	10.0	21.2	25.0	25.4	20.6
	Bb	15.1	10.9	...	18.1	25.0	19.7	15.2
	Cc	0.2
	Dd	1.1	4.5	5.0	0.8	2.4
	Ee	15.1	14.5	30.0	36.2	30.0	0.8	13.9
	Ff	0.4
	Gg	4.3	3.6	...	1.5	10.0	6.5	3.7
	Hh	14.1	14.5	30.0	31.8	30.0	0.8	12.6
	Ii	5.4	20.0	1.7	5.6
	Jj	5.4	20.0	1.7	5.6
	Kk	5.4	20.0	1.7	5.6
	Ll	5.4	20.0	1.7	5.6
	Mm	1.7	...
Mean per individual		2.13±0.13	1.65±0.14	1.60±0.38	2.18±0.17	3.20±0.46	1.66±0.09	2.00±0.06
Flies tested		92	55	10	66	20	192	461

The frequencies of the various inversions in the population samples studied are summarised in tables 3 and 4. It can be seen at a glance that the chromosomal variability is much greater in *D. guaramunu* than in *D. griseolineata*. In the former species, the localities in the State of Rio Grande do Sul (Chapada, Ponta Grossa, Itapoan and Feliz) have between 1.6 and 2.2 inversions per individual. In the State of São Paulo (Bragança, Mogí das Cruzes, Pirassununga) the inversion frequencies are about the same as in Rio Grande do Sul (1.7 to 3.2, the latter figure being subject to a large error). Since no inversions have been detected in the X-chromosome of these

species, the data for females and males can be combined. In *D. griseolineata*, the State of Rio Grande do Sul (Ponta Grossa, Itapoan, Emboaba) shows perhaps fewer inversions per individual than does the State of São Paulo (Vila Atlantica, Cantareira, and Mogí das Cruzes). But even the highest recorded inversion frequency per individual of *D. griseolineata* (0.47, cf. table 4) is lower than the lowest frequency found in *D. guaramunu* (1.6, cf. table 3).

According to Dobzhansky and Pavan (1950), *D. guaramunu* is more common and widespread in Brazil than is *D. griseolineata*. The former species proved to be one of the commonest species of *Drosophila* in 5 out of the 35 localities studied by these authors. The latter species

TABLE 4

Frequencies (in per cent.) of various inversions found in heterozygous condition, and the mean numbers of heterozygous inversion per individual of *D. griseolineata*

Chromosome	Inversions	Ponta Grossa	Itapoan	Emboaba	Vila Atlantica	Cantareira	Mogí das Cruzes
II	none	96.5	97.1	98.6	80.8	90.6	82.9
	<i>Aa</i>	1.1	...	2.6
	<i>Bb</i>	3.5	2.9	1.4	14.0	8.0	9.2
	<i>Cc</i>	4.1	1.4	5.3
III	none	94.7	100.0	100.0	86.4	81.0	86.8
	<i>Aa</i>	5.3	13.6	19.0	14.0
	<i>Bb</i>	5.3	13.2	19.0	14.0
Mean per individual		0.14 ± 0.06	0.02 ± 0.06	0.01 ± 0.04	0.47 ± 0.04	0.47 ± 0.09	0.46 ± 0.09
Flies tested		57	69	71	265	74	76

has been found in 6 out of the 35 localities, but in all of them it was one of the rarer forms. *D. guaramunu* is, indeed, one of the dominant species both in the savanna of central Brazil and in the subtropical forest of the State of Rio Grande do Sul. At Pirassununga, which represents ecologically a southern outlier of the savanna of central Brazil, the lowest frequency of *D. guaramunu* in 18 different collections made during four years of observation was about 2 per cent., and in 9 of these collections it was above 18 per cent., of the total catch of *Drosophila* flies. At Mogí das Cruzes, its frequency varied from 0 to 23 per cent. of the total, and at Vila Atlantica from 0 to 6 per cent. of the total. In Rio Grande do Sul, *D. guaramunu* is encountered with frequencies from 0.5 to 5 per cent. on the coast, and from 11 to 51 per cent. in the interior of the State.

D. griseolineata varies greatly in frequency, but it is on the whole much less common than *D. guaramunu*. The recorded frequencies of the former species vary from 0 to 30 per cent. at Vila Atlantica and

from 0 to 16 per cent. at Mogí das Cruzes. In Rio Grande do Sul, this species is relatively more common on the coast (2 to 30 per cent.) than in the interior, where it is quite rare. Furthermore, the frequencies of this species are subject to violent fluctuations at different seasons.

5. *DROSOPHILA NEBULOSA*

Drosophila nebulosa Sturtevant is a member of the *willistoni* species group of the subgenus *Sophophora*. Its distribution region extends from Texas in the North (Patterson and Wagner, 1943) to at least Buenos Aires in the South (Pavan, unpublished). No *Drosophila* collecting has ever been made south of Buenos Aires. In Brazil, *D. nebulosa* is one of the commonest species, particularly in drier savanna and desert regions, less common in the rain forests. Furthermore, the frequencies of *D. nebulosa* relative to those of other species of *Drosophila* tend to be higher during the warmer and drier than during the wetter and cooler seasons (Pavan, unpublished).

The metaphase plates of *D. nebulosa* show two pairs of metacentric and one pair of acrocentric chromosomes. Pavan (1946) examined the salivary gland chromosomes of a series of strains, and described 9 inversions, all but one of which were found in a single chromosome, the third, which is the acrocentric autosome. In our material, we have observed 7 out of the 8 inversions described by Pavan in the third chromosome, and found 3 new inversions, namely *Ii*, *Jj*, and *Kk*, also in the third chromosome. The single inversion found by Pavan in the X-chromosome was not found in our material.

The frequencies of the inversion heterozygosis in the populations examined by ourselves are given in table 5. Only the gene arrangements *HH*, *Hh* and *hh* were identified both when homozygous and when heterozygous. The inversions *Aa*, *Bb*, *Cc*, *Gg* and *Hh* occur frequently in all the regions studied; the inversions *Ee*, *Ff*, *Ii*, *Jj* and *Kk* are less common or restricted to only small fractions of the species distribution area.

It may be noted that a group of populations comprising Mucajaí, Monjolinho, Pirassununga, Iguassú and Tucuman show high frequencies of the gene arrangement *h*, while Rio Negro, Imperatriz, Salitre and Catuní are characterised by high frequencies of the alternative gene arrangement, *H*. This distribution of the frequencies of *h* and *H* shows no clear correlations with the ecological characteristics of the localities in which the respective samples were collected. Thus, Mucajaí is more similar to Monjolinho, some 2500 km. away, than it is to Rio Negro, less than 500 km. away. Mucajaí, Iguassú and Rio Negro are covered with rain forest, while the other localities have savanna or desert vegetation.

The mean frequencies of the numbers of heterozygous inversions per individual show, on the contrary, a fairly clear correlation with the ecological characteristics of the regions in which the population

TABLE 5

Frequencies (in per cent.) of various inversions found in heterozygous condition, mean number of heterozygous inversions per individual and relative frequencies (in per cent.) of *D. nebulosa* individuals in several populations

Third chromosome inversions	Mucajai	Rio Negro	Imperatriz Carolina	Salitre 1949	Salitre 1951	Catuní	Monjolinho	Pirasununga	Iguassú	Tucuman
<i>Aa</i>	38.5	40.0	32.4	40.0	33.0	20.0	32.3	8.7	8.0	6.0
<i>Bb</i>	43.6	54.3	8.1	4.0	3.0	12.7	26.1	8.7	30.0	36.0
<i>Cc</i>	48.7	42.8	32.4	32.0	21.0	7.3	58.5	34.8	27.0	...
<i>Ee</i>	4.6	...	1.0	...
<i>Ff</i>	5.4	4.3	4.0	...
<i>Gg</i>	41.0	42.8	2.7	11.0	3.0	...	26.1	26.1	24.0	23.0
<i>HH</i>	12.8	48.6	64.9	66.0	56.0	72.7	16.9	17.4	13.0	1.0
<i>Hh</i>	38.5	37.1	29.7	32.0	35.0	21.8	46.1	34.8	31.0	24.0
<i>hh</i>	46.1	14.3	5.9	2.0	9.0	6.5	36.9	47.8	56.0	75.0
<i>Ii</i>	23.0
<i>Jj</i>	16.2
<i>Kk</i>	2.5	14.3
Mean per individual	2.36 ± 0.19	2.29 ± 0.15	1.30 ± 0.16	1.20 ± 0.11	0.90 ± 0.10	0.62 ± 0.09	1.94 ± 0.12	1.17	1.25 ± 0.08	0.86 ± 0.08
Flies tested	39	35	37	100	100	55	65	23	100	100
Species frequencies	1.4-71.5	1.8	51-63	52.4	54.3	26.7	5.2-63.8	0.1-29	8.8	10.3

samples were taken. The highest frequencies are observed at Mucajai, Rio Negro, Monjolinho, and Imperatriz. The first and the second of these localities are covered with exuberant rain forests, but are close to margins of savannas. On Rio Negro in particular, *D. nebulosa* proved to be commoner in drier localities than in wetter ones (up to 50 per cent. of the total catch of *Drosophila* flies on the beach of Tatuauara, near Manaus). Monjolinho and Imperatriz lie in ecologically highly complex regions, on or near the ecotones between the savanna and the rain forest, or gallery forest, vegetations. By contrast, Salitre and Catuní, which show low frequencies of inversions, are in a desert (caatinga) region, which is dry and nearly lifeless during more than six months per year. Although *D. nebulosa* is the commonest species of *Drosophila* in this desert, it certainly can occupy there only a very limited number of ecological niches. It should be noted that *D. willistoni* has also shown the lowest numbers of heterozygous inversions per individual in this region (da Cunha, Burla and Dobzhansky, 1950). Finally, Tucuman lies at the foot of the Andes, presumably near the margin of the distribution area of the species. The population sample was collected there in an isolated forested region having a limited variety of plant species.

6. *DROSOPHILA BANDEIRANTORUM* AND *DROSOPHILA PALLIDIPENNIS*

Drosophila bandeirantorum Dobzhansky and Pavan and *D. pallidipennis* Duda are species with no clear affinities among the known species of *Drosophila*. The former has a rather restricted known geographic distribution area in southern Brazil. The latter has been recorded from the State of São Paulo, in southern Brazil, as well as from Mexico, but has so far not been encountered in the Amazon Basin. Both species are fairly rare, and appear to be specialised to occupy a restricted variety of ecological niches (Dobzhansky and Pavan, 1950). Their salivary gland chromosomes are among the best found in any species of *Drosophila*, and this served as a stimulus to study them for inversions.

In 441 examined individuals of *D. bandeirantorum*, two kinds of inversions were found, one in each, the second and the third chromosomes (autosomes). In *D. pallidipennis*, Patterson and Dobzhansky (1945), Freire-Maia and Engel (1949), and ourselves found two inversions, both of them in the same autosome (overlapping inversions). The mean frequency of heterozygous inversions per individual of *D. bandeirantorum* proved to be 0.49 ± 0.02 . The material examined came from 4 populations in the State of São Paulo. In the 70 individuals of *D. pallidipennis*, from Pirassununga, in the State of São Paulo, examined by Freire-Maia and Engel (1949) and ourselves, the mean number of heterozygous inversions per individual proved to be even smaller, namely 0.07 ± 0.04 .

7. DISCUSSION

The data presented above are in a general agreement with the hypothesis that the amount of genetic variability present in a population is positively correlated with the number of ecological niches which this population exploits. Thus, the common and widespread species, *D. polymorpha*, has more heterozygous inversions per individual than its closest but less common relative, *D. cardinoides*. Similarly, the common *D. guaranunu* has more inversions than its less frequent relative, *D. griseolineata*. In *D. nebulosa* the populations which inhabit the ecologically limited and highly exacting desert environments show fewer inversions than do the inhabitants of richer and more diversified rain forest and savanna environments (*cf.* da Cunha, Burla and Dobzhansky, 1950).

Several reservations must, however, be made. In the first place, only closely related species, or races within a species, can profitably be compared. In different groups of organisms the genetic variability takes different forms, and chromosomal inversions are characteristic of the adaptive polymorphism in some sub-divisions of the animal and plant kingdoms but not in others. Even within the genus *Drosophila*, some species groups seem to be richer in inversions than others (thus, the subgenus *Sophophora* seems to have more inversions than the subgenus *Drosophila*). Some species have inversions in all chromosomes (e.g. *D. willistoni* and relatives, *D. robusta*, *D. virilis*), while other species have them concentrated in a single or in few chromosomes (e.g. *D. pseudoobscura*, *D. persimilis*, *D. nebulosa*).

Furthermore, given the present imperfect understanding of the ecology of *Drosophila*, our ability to evaluate the relative numbers of the adaptive niches controlled by a population is limited. By and large, an abundant and widespread species is likely to be ecologically more versatile than a rare one restricted to only few habitats. It stands, however, to reason that an ecologically specialised species may occur more commonly than an ecologically versatile one, provided that the special environment of the former is available in many places within a certain geographic region. Situations apparently contradictory to the hypothesis are, then, not unexpected, but, provided that the hypothesis is correct, they should be less frequent than situations conforming to the hypothesis. Such an apparent contradiction may be seen in table 1. *D. polymorpha* has significantly more inversions per individual at Vila Atlantica than at Pirassununga or at Mogí das Cruzes. And yet this species reaches at times much higher frequencies at the latter than at the former localities. It may be significant in this connection, that the population size undergoes violent fluctuations at Pirassununga and at Mogí das Cruzes, reaching up to 22 per cent. of the total *Drosophila* catch during the summer, but becoming very rare during winter. At Vila Atlantica the population of *D. polymorpha* does not undergo such sharp expansions and contractions at different seasons.

8. SUMMARY

Natural populations of several species of *Drosophila* were examined for the incidence of inversion heterozygotes among the individuals composing them. Inversion heterozygotes proved to be relatively more frequent in *Drosophila polymorpha* than in its close relative, *Drosophila cardinoides*, and more frequent in *Drosophila guaramunu* than in the neighbouring species, *Drosophila griseolineata*. *Drosophila polymorpha* and *Drosophila guaramunu* are more common and widespread in southern Brazil than are *Drosophila cardinoides* and *Drosophila griseolineata*. In *Drosophila nebulosa*, populations which inhabit ecologically rich and diversified environments of savannas and rain forests have more inversions than do geographically or ecologically marginal populations. These facts stand in accord with the hypothesis that the amount of adaptive polymorphism present in a population is positively correlated with the variety of environments which this population controls.

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