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EXPERIMENTS ON SEXUAL ISOLATION IN SPECIES OF THE
MESOPHRAGMATICA GROUP OF *DROSOPHILA* (DIPTERA)¹

By SUSI KOREF SANTIBAÑEZ, ADRIANA CASANOVA AND DANKO BRNCIC

(*Catedra de Biología, Escuela de Medicina, Universidad de Chile*)

[Communicated by E. B. Basden, A.R.C. Unit of Animal Genetics,
 Institute of Animal Genetics, Edinburgh]

INTRODUCTION

UP to now the following six species of the *mesophragmatica* group of the genus *Drosophila* are known: *D. mesophragmatica* Duda, 1925; *D. gaucha* Jaeger and Salzano, 1953; *D. pavani* Brncic, 1957; *D. altiplanica* Brncic and Koref, 1957; *D. orkui* Brncic and Koref, 1957; and *D. viracochi* Brncic and Koref, 1957. Although most of these species are allopatric for characteristic regions in South America, in several places their distribution areas overlap. Thus, *D. viracochi*, *D. mesophragmatica* and *D. orkui* are sympatric in Peru (Cuzco); *D. mesophragmatica*, *D. altiplanica* and *D. gaucha* are all found together in Bolivia, and *D. pavani* (a typically Chilean species) and *D. gaucha* overlap in a small zone in Argentine (province of San Luis).

All these species can be considered as separate biological entities, and can be distinguished by slight morphological differences, with some, as *D. pavani* and *D. gaucha*, or *D. altiplanica* and *D. orkui*, constituting cryptic (sibling) species (Brncic and Koref, 1957a). In previous papers by these authors it was shown that the natural populations of these six species are reproductively isolated. There is no gene exchange between them, even in the regions where they are found together.

This fact is not new to those who are familiar with the taxonomy, genetics and evolution of the genus *Drosophila*. Patterson and Stone in their revision (1952, ch. 7) point out several cases of species which are morphologically very similar or even indistinguishable, but which nevertheless represent closed genetic systems, according to our biological concept of species (Dobzhansky, 1951; Mayr, 1942). These systematic entities are important. On the one hand, it may be possible to clarify several evolutionary problems if one is able to know the mechanisms that take part in this incipient stage of speciation, based on the lack of gene exchange. On the other hand, as has been pointed out by Dobzhansky (1951, 1956) and others, the existence of cryptic species proves that morphological differentiation does not necessarily go together with evolutionary divergence.

This lack of gene exchange between different species is the result of several types of isolating mechanisms. These are usually different for each group of species, and in general one alone is not sufficient to prevent the production of hybrids.

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The object of the experiments mentioned in this paper was to study some of the mechanisms responsible for sexual isolation between the members of the *mesophragmatica* group.

MATERIAL AND METHODS

The following were the stocks used: *D. mesophragmatica* from Cuzco (Peru) and La Paz (Bolivia); *D. gaucha* from Rio Grande do Sul (Brazil), Sao Paulo (Brazil) and Cordoba (Argentina); *D. parani* from Copiapo (Chile), Vellenar (Chile), Santiago (Chile) and Mendoza (Argentina); *D. altiplanica* from La Paz (Bolivia); *D. orkui* from Cuzco (Peru) and *D. viracochi* from Machu Pichu (Peru).

The single choice mating method (*i.e.* one species of male with one species of female) was used to investigate the different sexual isolating mechanisms between the species of the *mesophragmatica* group. The morphological features, the chromosomes and the phylogenetic relationships of these six species have already been analysed in previous papers of Brncic and Koref (1957*a*, 1957*b*). One or several one- to two-week-old virgin females from the above-mentioned stocks were placed in separate vials with an equal number of males of either the same or of a different species. After a delay of about four weeks, due to the slow development of the flies, the females were dissected for the detection of sperm in their ventral receptacles and spermathecae.

All matings were done in 10 × 3 cm. vials with the usual *Drosophila* food medium, and during the course of the experiment were maintained at a mean temperature of 18° C.

RESULTS

The results of the 36 crosses studied are summarised in Table I. As no appreciable differences could be seen between the different stocks of each species, their data have been combined.

Progeny were obtained in all six intraspecific crosses, but only in six of the 30 possible interspecific ones. The heterogamic matings which were most successful were those between *D. gaucha* and *D. parani*, where an abundant adult F₁ was obtained. These results are analysed farther on.

In the crosses *D. altiplanica* ♀ × *D. orkui* ♂,¹ as well as in the reciprocal ones, a few F₁ flies emerged. The hybrid males had atrophied testes and were totally sterile. Only a few of the females, in spite of having apparently normal ovaries, gave any progeny when back-crossed to either *D. altiplanica* or *D. orkui* males.

In the crosses *D. mesophragmatica* ♀ × *D. parani* or *D. gaucha* ♂ the few hybrids obtained developed only up to the pupal stage.

It is interesting that in those crosses in which no progeny were obtained, the females had only non-motile sperm in their genital tract. The percentage of

¹The usual zoological practice is to consider the sexes in the order male-female, not female-male; and on hybrids the International Code of Zoological Nomenclature has a definite ruling (Article 18). "The notation of hybrids may be given in several ways: in all cases the name of the male parent precedes that of the female parent, with or without the sexual signs". Most *Drosophilists*, however, through following A. H. Sturtevant (1921, *Genetics* 5 (5): 488-500), Th. Dobzhansky, and the Texas School have habitually placed the female parent before the male parent. The new Zoological Code may amend the present ruling (*v.* 1957, *Bull. zool. Nomen.* 14: 122). --E. B. B.

females with non-motile sperm indicated in Table I is only approximate, as under this column several different situations have been included, from those with abundant sperm, easy to diagnose under the microscope, to those females

TABLE I. *The production of hybrids in various crosses between members of the mesophragmatica group of Drosophila*

Males	Females	Females dissected	Large amount of motile sperm (%)	Small amount of motile sperm (%)	Non-motile sperm (%)	Absence of sperm (%)	Hybrids produced
<i>mesoph.</i>	<i>mesoph.</i>	100	77.00	10.00	9.00	4.00	Fertile ♂♂, ♀♀
	<i>altipl.</i>	34	.	.	85.29	14.7	None.
	<i>gaucha</i>	110	.	.	75.45	24.54	..
	<i>orkui</i>	52	.	.	100.00	.	..
	<i>parani</i>	87	.	.	63.21	36.78	..
	<i>viraco.</i>	84	.	.	65.47	34.52	..
<i>altipl.</i>	<i>altipl.</i>	100	82.00	10.00	5.00	3.00	Fertile ♂♂, ♀♀
	<i>gaucha</i>	37	.	.	89.18	10.81	None.
	<i>mesoph.</i>	60	.	.	93.33	6.66	..
	<i>orkui</i>	62	6.45	22.58	64.51	6.45	Few sterile ♂♂, ♀♀*
	<i>parani</i>	58	.	.	62.09	37.93	None.
	<i>viraco.</i>	29	.	.	55.17	44.82	..
<i>gaucha</i>	<i>gaucha</i>	100	80.00	3.00	12.00	5.00	Fertile ♂♂, ♀♀
	<i>altipl.</i>	32	.	.	93.75	6.25	None.
	<i>mesoph.</i>	88	.	2.27	78.40	19.31	Only few pupae.
	<i>orkui</i>	46	.	.	93.47	6.52	None.
	<i>parani</i>	120	82.50	12.50	1.66	3.33	Mostly sterile ♂♂, ♀♀
	<i>viraco.</i>	38	.	.	57.89	42.10	None.
<i>orkui</i>	<i>orkui</i>	82	84.14	12.19	2.43	1.22	Fertile ♂♂, ♀♀
	<i>altipl.</i>	51	13.72	3.92	78.43	3.92	Few sterile ♂♂, ♀♀*
	<i>gaucha</i>	47	.	.	72.34	27.65	None.
	<i>mesoph.</i>	59	.	.	59.32	40.67	..
	<i>parani</i>	52	.	.	69.23	30.76	..
	<i>viraco.</i>	68	.	.	67.64	32.35	..
<i>parani</i>	<i>parani</i>	104	73.07	2.88	23.07	0.96	Fertile ♂♂, ♀♀
	<i>altipl.</i>	35	.	.	45.71	54.28	None.
	<i>gaucha</i>	109	76.14	14.67	5.50	3.66	Sterile ♂♂, ♀♀
	<i>mesoph.</i>	53	.	1.88	81.13	16.98	A few pupae.
	<i>orkui</i>	45	.	.	80.00	20.00	None.
	<i>viraco.</i>	42	.	.	50.00	50.00	..
<i>viracochi</i>	<i>viraco.</i>	100	76.00	.	22.00	2.00	Fertile ♂♂, ♀♀
	<i>altipl.</i>	18	.	.	50.00	50.00	None.
	<i>gaucha</i>	90	.	.	77.77	22.22	..
	<i>mesoph.</i>	54	.	.	64.81	35.18	..
	<i>orkui</i>	28	.	.	25.00	75.00	..
	<i>parani</i>	99	.	.	63.63	36.36	..

* Some ♀♀ later found to be fertile to either parent male.

which had only very few sperm. The latter cases were most frequent, and there may be quite a range of error due to individual subjective appreciation. Nevertheless, it is clear that there is copulation with insemination between all members of the six species, and in those cases in which no progeny is obtained, the heterologous sperm are speedily inactivated within the reproductive tract of the female. The results seem similar to some cases recorded by Patterson (1954) in crosses between members of the *virilis* group. The insemination reaction (Patterson and Stone, 1952, ch. 8), frequent in other *Drosophila* species-groups, was not seen in the *mesophragmatica* complex.

In the crosses from which hybrids were obtained, a certain number of the parent females showed motile sperm in their ventral receptacle. The percentage of females inseminated, as well as the number of motile sperm found, seem to be in direct relation to the abundance and viability of the progeny observed. Only in *D. pavani* and *D. gaucha* was the number of inseminated females greater in the heterogamic than in the homogamic matings.

As both hybrid males and females from the crosses between the sibling species *D. gaucha* and *D. pavani* showed apparently normal gonads, the fertility of the progeny was tested. The F_1 sexually mature females were mated individually, or in small mass cultures, to an equal number of F_1 males. In the case of the mass cultures in which larvae could be seen, the females were placed separately in individual vials. The same procedure was used for the back-crosses. Table II summarises some of the results obtained.

TABLE II.—*Hybrids between D. gaucha and D. pavani*

Crosses	P × P	F ₁ × F ₁	♂ F ₁ Back-crosses		♀ F ₁ Back-crosses		
			With ♂ <i>gaucha</i>	With ♀ <i>pavani</i>	With ♀ <i>gaucha</i>	With ♂ <i>pavani</i>	
<i>gaucha</i> ♀ × <i>pavani</i> ♂							
Number of crosses	50	318	33	36	30	30	
Percentage with progeny	76.00	0	0	0	0	0	
<i>pavani</i> ♀ × <i>gaucha</i> ♂							
Number of crosses	88	110	37	31	30	32	
Percentage with progeny	79.54	3.63	24.32	0	20.00	9.37	

The first fact is that both male and female hybrids from *gaucha* ♀ × *pavani* ♂ crosses are completely sterile, not only when mated to each other but also when back-crossed to either parent. On the other hand, some male and female progeny from the reciprocal cross, *pavani* ♀ × *gaucha* ♂, are fertile, a few individuals being obtained from the back-crosses (except with *pavani* ♂) and from the crosses between them. It is interesting that only non-motile or no sperm are ever found in the reproductive tract of the hybrid females from *D. gaucha* ♀ × *D. pavani* ♂, whereas in females coming from the reciprocal cross a certain number of live sperm was observed.

These contrasting results cannot be explained only by chromosomal differences, as the hybrid females from both reciprocal crosses have the same number of chromosomes from *D. pavani* and from *D. gaucha*. This suggests the existence of some cytoplasmic or maternal influence in the sexual isolation between these

two species, as has already been found in other members of the genus *Drosophila* (cf. Patterson and Stone, 1952, ch. 10).

DISCUSSION

As has been pointed out by Dobzhansky (1951) and by Patterson and Stone (1952, ch. 7), there are several isolating mechanisms in the genus *Drosophila*. Some of these, such as geographic, ecological or seasonal isolation, can only be discovered by a deep study of the dynamics of the natural populations, while others, such as reproductive isolation, may be investigated in the laboratory. According to the above authors, the reproductive isolating mechanisms, which can be studied experimentally, may be classified as follows: (1) mechanical isolation due to differences in genitalia; (2) sexual or psychological isolation or failure to mate; (3) insemination reaction and perhaps failure to inseminate females sufficiently; (4) death or sterilisation of cross-mated individuals; (5) gamete mortality; (6) zygote or hybrid mortality; and (7) zygote or hybrid sterility.

In the *mesophragmatica* group it seems clear that some of the mechanisms (3)–(7) play an important part in preventing genetic exchange between the species. In all crosses, except in those between *D. gaucha* and *D. pavani*, lack of sufficient insemination and gamete mortality were found, due to the small amount of sperm, of which little if any was motile in the genital tract of the females.

Moreover, in the crosses *D. mesophragmatica* ♀ × *D. gaucha* or *D. pavani* ♂, there was an early death of the zygotes, as the hybrids developed only up to the pupal stage. The zygote mortality may be due to several causes, all related to a genetic incompatibility. The cytological study of the giant chromosomes of hybrid larvae (Brncic and Koref, 1957b) shows that the genetic arrangement along the chromosome of one species differs from that of the other species by many inverted zones, and regions in which there is lack of coupling.

In the crosses between *D. orkui* and *D. altiplanica* the above factors, and also partial hybrid sterility, have been observed. This sterility is undoubtedly based on genetic incompatibility, as the hybrid chromosomes show a great number of inversions; it also has an anatomic expression in the atrophied testes of the hybrid males. The females, on the other hand, have apparently normal gonads, but only some of them give progeny when back-crossed to either *D. orkui* or *D. altiplanica*.

Although the chromosome configuration of *D. altiplanica* places it nearer to *D. gaucha* and to *D. viracochi* (Brncic and Koref, 1957b), its degree of reproductive isolation brings it nearer to *D. orkui*. In nature up to now they have been found in separate regions (*orkui* in Peru and *altiplanica* in Bolivia), but they may yet be found to overlap in some zone.

The most subtle, but not the least effective, reproductive isolating mechanisms are those found between *D. gaucha* and *D. pavani*. In Table I, it is possible to see that the number of inseminated females and of those with motile sperm is even greater in the crosses between species than in the homogamic ones. On the other hand, the hybrids are less fertile, even though anatomically both male and female gonads are apparently normal. Hybrids

copulate, but the sperm are inactivated, except in some matings between *D. pavani* females and *D. gaucha* males from which a few progeny are obtained (Table II). The difference in results between these and the reciprocal crosses (*D. gaucha* ♀ × *D. pavani* ♂) may indicate the presence of some cytoplasmic or maternal factor responsible for the incompatibility.

Both *D. pavani* and *D. gaucha* males, when crossed with the five other species, give very similar results (Table I). When crossed with *D. mesophragmatica* females non-viable pupae are produced. These results, together with the other factors analysed, the similar external appearance and chromosome configuration of the two species (Brncic and Koref, 1957b), further suggest that they are very closely related from an evolutionary standpoint, and also more related to *D. mesophragmatica*, or at least to the female of that species, than to other members of the group.

Finally, unpublished data of Brncic and Casanova indicate that there is still another element in the reproductive isolation between these two species. With the multiple choice technique, in which males of one species are placed with females of both, it is seen that the males exhibit a tendency to copulate with females of their own species. This shows the existence of a certain degree of psychological isolation. It may explain in part the absence of hybrids in nature in the regions where both species are sympatric.

From what has been discussed, it can be seen that many reproductive isolating mechanisms operate between the members of the *mesophragmatica* group, and though they are different for each of the species, their final result is to prevent gene exchange which may cause a breakdown of the genetic systems built up by each species.

SUMMARY

By means of the single choice mating method it was shown that between the six known species of the *mesophragmatica* group of the genus *Drosophila*, *D. mesophragmatica*, *D. gaucha*, *D. pavani*, *D. altiplanica*, *D. orkui* and *D. viracochi*, there are varying degrees of reproductive isolation. Although in all crosses males and females copulate, in most heterogamic matings the sperm are rapidly inactivated within the genital tract of the females.

In both reciprocal crosses *D. altiplanica* × *D. orkui* a few males with atrophied testes and mostly sterile females with apparently normal ovaries are obtained. From the crosses *D. mesophragmatica* ♀ × *D. pavani* or *D. gaucha* ♂, the few hybrids develop only to the pupal stage. Only in matings between *D. pavani* and *D. gaucha* is an abundant F₁ obtained, most of which hybrids are sterile, except a few males and females from crosses *D. pavani* ♀ × *D. gaucha* ♂. In the reciprocal cross 100 per cent. of the hybrids are sterile. These differences suggest the intervention of certain maternal or cytoplasmic factors.

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