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NOTES AND COMMENTS

HYBRID STERILITY IN *DROSOPHILA SIMULANS*: RELATIONSHIPS WITH THE HYBRID DYSGENESIS SYNDROME IN *DROSOPHILA MELANOGASTER*

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1. Introduction

In recent years, several independent lines of research have led to the discovery that high levels of sterility may occur within the *Drosophila melanogaster* species as a result of crosses between different strains (Kidwell *et al.*, 1977; Picard *et al.*, 1978; Angus and Raisbeck, 1979; Engels and Preston, 1979) or as a result of intrastrain expressivity (Périquet, 1978, 1980). Two systems of hybrid dysgenesis have been described—the *P-M* system, the subject of the present report, and the *I-R* system. In the former, hybrid sterility is characterized by gonadal dysgenesis (GD sterility), the ovaries and testes being atrophied unilaterally (S1 type) or bilaterally (S0 type).

The fact that *D. melanogaster* exhibits at least two systems of hybrid dysgenesis has led many authors to deduce a possible occurrence of hybrid dysgenic systems among other species (see review in Bregliano *et al.*, 1980). The purpose of this study is to explore the possibility of hybrid dysgenesis in *D. simulans*, mainly by investigating the gonadal dysgenesis phenomenon, and to discuss implications about the evolution of the *P-M* system from the results obtained.

2. Materials and methods

Hybrid dysgenesis in the *P-M* system can be recognized by its reciprocal cross effect that is, most strains can be classified as either *P* (Paternal contributor) or *M* (Maternal contributor), such that hybrids from crosses of the form $M♀ \times P♂$ (called cross A) show dysgenic traits which are reduced or absent in the reciprocal hybrids from cross B.

(i) *Wild strains employed*

The wild-type strains used were mainly collected by David and Tsacas and kept by mass culture at 20°C in the laboratory on David's axenic medium. Eight French strains collected during the 1970s were used: Menton 71 (Men), Narbonne 71 (Nar), Sainte Maxime 71 (SMx), Garat 76 (Gar), Hyères 76 (Hyr), Prat 76 (Prt), Gif-mare 77 (Gif), Villeurbanne 77 (Vil) and 2 African strains: Salazie 75 (Sal) in Reunion Island and Bugarama 79 (Bug) in Burundi.

(ii) *Determination of GD sterility*

Crosses between 30 males and 30 females of the different strains were set up and immediately placed in a 28°C incubator. The parents were removed after 2-3 days. Approximately 100 F_1 females were determined for GD sterility. Ovaries and testes of the 3-5 day old imagoes were dissected in Ringer solution and classified as S0, S1 or S2 types. It has been demonstrated that for GD sterility there is normally a close relationship between the frequency of sterility as determined by the absence of eggs and the frequency of atrophic dysgenic ovaries after dissection (Kidwell and Novy, 1979; Périquet, 1980). Almost all crosses were duplicated in a separate experiment.

3. Results

(i) *Crosses among strains*

The results of female sterility tests for many inter and intrastrain cross combinations are presented in table 1. The characteristic of most interacting

TABLE 1

Mean percentage of female gonadal dysgenesis in 10 strains of D. simulans *and in the progeny of reciprocal crosses among them. All flies were raised at 28°C and at least 100 females per cross were dissected. The first number is the S1 type percentage and the second the S0 type percentage*

	Males									
Females	Nar	Bug	Sal	Prt	SMx	Gar	Men	Vil	Gif	Hyr
Nar	4-50	2-2	4-19	8-17	2-24	10-20	5-34	10-39	2-0	1-1
Bug	0-2	2-12	0-0	4-17	· ·	2-18	6-26	2-15	· ·	0-0
Sal	2-1	0-0	3-0		0-0	0-0	· ·	0-5	· ·	0-4
Prt	4-0	0-0	· ·	4-6	5-5	0-0	1-4	0-4	0-0	0-0
SMx	0-0	· ·	0-0	0-0	0-3	0-0	0-0	0-0	· ·	0-0
Gar	0-1	0-0	0-0	0-0	0-0	0-0	0-0	· ·	· ·	· ·
Men	0-0	0-0	· ·	2-5	0-0	0-0	0-2	1-5	1-1	0-0
Vil	1-1	0-0	0-0	1-0	0-0	· ·	1-4	5-25	0-2	· ·
Gif	0-0	· ·	· ·	0-0	· ·	· ·	0-0	0-2	1-2	0-0
Hyr	0-0	0-0	0-0	0-2	0-0	· ·	0-0	· ·	0-0	0-0

strain crosses observed in this table is the large difference in the frequencies of gonadal dysgenesis between the progeny of reciprocal strain crosses. In order to describe this non-reciprocity, it is convenient to designate strains with the notation used for *Drosophila melanogaster*, as either *P* or *M*.

The tested strains appear unambiguously as either of the *M* type (Narbonne 71 and Bugarama 79) or of the *P* type (Menton 71, Sainte Maxime 71, Salazie 75, Garrat 76 and Villeurbanne 77), but can also fall into a third category, neutral type *Q* (Hyères 76 and Gif 77), which does not react with either *P* or *M* strains. Strains of the same *M* or *P* type present similar properties with respect to gonadal dysgenesis, but may differ in the intensity of interaction. Intrastrain sterility, shown on the diagonal, presents considerable variation among the strains. No close relation appears between the frequency of intrastrain sterility and the type of strain. High and low levels of intrastrain sterility are encountered both in the *M* (Narbonne 71, 50 per cent, Bugarama 79, 12 per cent) and *P* strains (Villeurbanne 77, 25 per cent, Menton 71, 2 per cent). Nevertheless, *Q* strains seem to present only a low frequency of intrastrain sterility. These

results agree with those obtained in *D. melanogaster*. Although the first observations in this species did not show intrastrain sterility above the ten percent level at 25°C (Kidwell *et al.*, 1977), it should be pointed out that intrastrain sterility also appears in the reference *P-M* strains of *D. melanogaster*, when more than 2 generations are raised at 28°C (Périquet, 1980). Moreover, experiments with *D. melanogaster* reported elsewhere (Périquet, 1980) showed that the sterility of strains presenting different levels of "atrophie gonadique" does not directly depend upon a specific type in the *P-M* system and may be associated either with the *M* type or the *P* type.

Analysis of table 1 shows that, as for *D. melanogaster*, the classification might hold for crosses of strains of different geographical origin. In the French strains tested, one falls in the *M* class and four in the *P* class, whereas with the African strains one is *M* and the other *P*. Furthermore, even if the number of tested strains is too small to determine whether the old laboratory strains are more often of the *M* type as in *D. melanogaster*, the case of the Bugarama strain is interesting. This strain has been tested only six months after its arrival at the laboratory and reveals an *M* activity. If it has not switched classes during this six month interval, this would be the first observation of an *M* strain in the wild.

(ii) *Temperature interaction*

Previous experiments with *D. melanogaster* indicate that high temperature (above 27°C) has a great effect on the manifestation of gonadal dysgenesis (Kidwell and Novy, 1979). In *D. simulans* a series of reciprocal crosses between Narbonne 71, Narbonne 71-plum (a homozygous line for *pm* (II, 103) isolated from Narbonne 71), Bugarama 79, Menton 71 and Villeurbanne 77 were set up. Mass matings were made in bottles and placed in incubators kept at temperatures ranging from 20 to 28°C. At least 200 F_1 females and 200 males from each treatment group were dissected. The results are presented in table 2. From cross A and in both sexes, sterility

TABLE 2

Percent of F_1 gonadal dysgenesis in reciprocal strain crosses raised at four developmental temperatures. The first number is the S1 type percentage, the second the S0 type percentage. At least 200 ♀ and 200 ♂ were dissected at each temperature; all values obtained at 20°C were nil and thus are not reported here

		Cross A			Cross B		
F_1 progeny		28°	25°	23°	28°	25°	23°
Nar × Vil	♀	10-39	3-1	0-0	1-1	0-0	0-0
	♂	3-38	2-1	—	0-0	0-0	—
Nar (pm) × Vil	♀	14-32	1-2	—	0-0	0-0	—
	♂	4-35	0-2	—	0-0	0-0	—
Nar × Men	♀	5-34	2-0	0-0	0-0	0-0	0-0
	♂	9-30	2-0	—	0-0	0-0	—
Nar (pm) × Men	♀	13-36	1-0	—	0-1	0-0	—
	♂	18-41	2-0	—	0-0	0-0	—
Bug × Vil	♀	1-22	1-0	0-0	0-0	0-0	0-0
	♂	3-24	0-1	—	0-0	0-0	—
Bug × Men	♀	6-26	1-1	0-0	0-0	0-0	0-0
	♂	2-23	1-0	—	0-0	0-0	—

increased with temperature, but was as high in females as in males, which is not the case in *D. melanogaster* where sterility is higher in females. Below 26-27°C, practically no gonadal atrophy was observed in F_1A nor in the control reciprocal cross (F_1B).

Analysis of the relationship between sterility and temperature can use the theoretical models proposed in the case of penetrance of the "atrophie gonadique" character in *D. melanogaster* (Périquet, 1978). In these models we assume that the inability of gonads to develop normally is attributable to the inefficiency of the pole cells. This hypothesis is similar to that proposed to explain sterility in hybrid dysgenesis where it is suggested that gonadal dysgenesis is caused by a failure in the early development of germ cells (Engels and Preston, 1979). In the simplest model it is assumed that each germ cell can be killed independently of the others during heat treatment and that an $S1$ type individual requires the survival of at least one pole cell to create a fertile gonad; in the case of several surviving pole cells, these would be randomly distributed between the two primordial gonads. They form either an $S2$ individual or, if all cells are included in the same gonad, an $S1$ individual. Hence the following distribution would account for the expected frequency of gonadal types:

$$\overline{S0} = e^{-s}$$

$$\overline{S1} = \sum_{n=1}^{\infty} \left[(1/2)^{n-1} \cdot e^{-s} \cdot \frac{s^n}{n!} \right]$$

$$\overline{S2} = 1 - S1 - S0$$

with s as the mean number of surviving pole cells per individuals. This corresponds to the binomial model tested by Engels and Preston (1979). From the definition of $\overline{S1}$ we have: $\overline{S1} = [2E(2^{-n}) - e^{-s}]$, where the expectation $E(2^{-n}) = e^{-s/2}$ is based on the Poisson distribution, thus $\overline{S1} = 2e^{-s/2}(1 - e^{-s/2})$, with the estimate of maximum likelihood: $\hat{s} = -\log_n (N0 + N1/2)^2$, where $N0$ and $N1$ are the observed relative frequency of the $S0$ and $S1$ classes (Engels, personal communication). Haldane's exact test shows a significant deviation ($P < 0{\cdot}01$) from the expectation which is due to an excess of those frequencies in the $S0$ and $S2$ classes of both sexes. These results are in accordance with those found in *Drosophila melanogaster*, where the same significant low frequency of unilateral ($S1$) individuals is found both in gonadal dysgenesis and in the "atrophie gonadique" character. Developmental temperature thus appears to have a very similar action on the origin and the manifestation of gonadal dysgenesis in both species. The hypothesis of gonadal dysgenesis caused by a lack of development of the germ cells can also explain the relative lack of male sterility in *D. melanogaster*. Since male embryos have approximately three times more germ cells in their gonads than females (Sonnenblick, 1950), the chance of at least one cell surviving is great (Périquet, 1978; Engels and Preston, 1979). In *D. simulans*, the observation of the same level of sterility among sexes may be explained by an almost equal number of germ cells in male and female embryonic gonads.

(iii) *Transmission ratio distortion*

In *D. melanogaster*, a transmission distortion effect has been found in dysgenic males. A comparison of the ratios between A and B type crosses

have shown that F_1 dysgenic males give distorted ratios for both chromosomes II and III in the A cross as compared to the B cross (Kidwell *et al.*, 1977). In addition, sex ratio distortion was also found to be part of hybrid dysgenesis (Engels, 1979).

To test the possibility of such a phenomenon in *D. simulans*, Mass A and B crosses between Narbonne 71-plum (*M* strain) and Menton 71 (*P* strain) were made in bottles and maintained at 25 and 28°C. F_1 males and F_1 females were respectively backcrossed to Narbonne 71-plum flies for all crosses, their F_2 progeny scored and the *K* ratio = [(number of + offspring)/(Total progeny offspring)] calculated. The results (table 3) show

TABLE 3

Proportions of progeny classes from back-crosses between F_1 Nar (pm)/Men males or females and the Nar (pm) (reference strain)

F_2 progeny from		28° pm	28° +	28° Total progeny	25° pm	25° +	25° Total progeny
♀F_1 (A) Nar (pm)/Men × ♂ Nar (pm)	♀	0·484	0·516	649	0·513	0·487	550
	♂	0·464	0·536	646	0·491	0·509	576
♂F_1 (A) Nar (pm)/Men × ♀Nar (pm)	♀	0·467	0·533	580	0·484	0·516	614
	♂	0·484	0·516	558	0·524	0·476	569
♀F_1 (B) Men/Nar (pm) × ♂ Nar (pm)	♀	0·509	0·491	1042	0·519	0·481	669
	♂	0·504	0·496	908	0·471	0·529	714
♂F_1 (B) Men/Nar (pm) × ♀Nar (pm)	♀	0·515	0·485	462	0·500	0·500	338
	♂	0·522	0·478	448	0·476	0·524	368

Heterogeneity test within A crosses, 28°C: $\chi^2 = 0{\cdot}81$, 3 d.f., $P = 0{\cdot}85$; 25°C: $\chi^2 = 2{\cdot}41$, 3 d.f., $P = 0{\cdot}50$; within B crosses, 28°C: $\chi^2 = 0{\cdot}44$, 3 d.f., $P = 0{\cdot}92$; 25°C: $\chi^2 = 3{\cdot}70$, 3 d.f., $P = 0{\cdot}40$; between A and B crosses, 28°C: $\chi^2 = 6{\cdot}73$, 1 d.f., $P < 0{\cdot}01$; 25°C: $\chi^2 = 0{\cdot}51$, 1 d.f., $P = 0{\cdot}50$.

that some segregation distortion occurred at 28°C in F_2 progeny from A crosses but not in F_2 progeny from B crosses. Among A crosses as among B crosses, no significant difference is shown in the progenies of F_1 males as compared to those of F_1 females. Significant differences only appear when the progenies of the reciprocal crosses are compared; the frequency of the *plum* chromosome is reduced in the A cross as compared to the B cross. At 25°C, no significant segregation distortion in F_2 progenies from any cross appears. For sex ratio distortion, the proportion of sons produced at 28°C by the A cross (49 per cent) is slightly but not significantly ($P = 0{\cdot}13$) higher than that of the B cross (47 per cent), and no significant distortion appears at 25°C.

The present data suggest that an effect of transmission ratio distortion correlated with hybrid dysgenesis may exist in *D. simulans*. With the strain employed this effect is weak and only becomes significant at 28°C. Such an effect of increasing temperature from 25 to 28°C on transmission ratio is in agreement with the data of Kidwell and Novy (1979), but differs from those of Engels (1979) who observed a 25°C maximum for *k* values. A more striking difference appears with *D. simulans* in the fact that the *k* ratio would be inverted, *i.e.*, the chromosome of paternal origin (the wild one here) presents a relative increase in its frequency, whereas it is reduced in the case of *D. melanogaster*.

4. Discussion

The existence of hybrid dysgenesis raises the question of the importance of such a phenomenon to population biology and the evolution of Drosophila. In an approach to these questions, extensive surveys on wild and laboratory strains have been conducted in order to classify populations in both *P-M* and *I-R* systems (Picard *et al.*, 1978; Kidwell, 1979). Results show that strains of *D. melanogaster* recently taken from nature are usually *IP*, whereas laboratory strains may either be *IM* or *RM*. Another approach has been to study a particular population of *D. melanogaster* intensively (Engels and Preston, 1980). In the wild Wisconsin population these authors found the *P* factor to be very common, but the *M* cytotype present at only very low frequencies; such a situation probably represents a stable condition with dysgenic traits occurring rarely if at all.

The results presented above clearly indicate that dysgenic traits also appear in *D. simulans* with large frequency differences between the progeny of reciprocal strain crosses. Gonadal dysgenesis, which is the trait mainly observed here, presents the same pattern as in *D. melanogaster* with respect to the non-reciprocity of occurrence and temperature interaction. The action of temperature shows that an early failure in the development of primordial germ cells is likely.

The presence of intrastrain sterility in different strains of *D. simulans* might appear at variance, with the idea that dysgenic traits do not normally occur within homogeneous strains. In *D. melanogaster*, however, observations of gonadal atrophy in reference *P* and *M* strains and striking similarities between intrastrain sterility and the "atrophie gonadique" character found in natural populations, suggest that gonadal dysgenesis can sometimes occur naturally and that these strains cannot be considered as homogeneous *P* or *M*, but present some degree of polymorphism (Engels and Preston, 1980; Périquet, 1980).

The second dysgenic trait observed in *D. simulans*, transmission ratio distortion, also appears to be correlated with the class of crosses but only at 28°C. These results must be confirmed in other crosses.

A third important dysgenic trait, highly correlated with hybrid dysgenesis in *D. melanogaster*, is male recombination. Unfortunately, efforts to test this trait in our experiments were unfruitful because the multimarked stock available in our laboratory proved to be neutral when tested with the reference lines. It must be remembered that in *D. simulans* spontaneous recombination in males has been shown (Woodruff and Bortolozzi, 1976) although its mechanisms may vary.

In conclusion, a phenomenon of gonadal dysgenesis exists in *D. simulans* which presents striking similarities to the corresponding trait in *D. melanogaster*. The marked difference between wild populations and most laboratory strains in *D. melanogaster* has suggested that one of these groups may have recently changed. Kidwell (1979) postulates that it is the wild populations that have changed under the rapid spread of the *P* factor through chromosomal contamination (Picard, 1978), assuming this sometimes occurs in the *P-M* system (Yannopoulos, 1979). The analysis of hybrid dysgenesis in *D. simulans* might give information to help answer questions on the evolutionary relations within species and between sibling species.

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