Differences in the cytotype and hybrid dysgenesis inducer ability of different P-strains of *Drosophila melanogaster*

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Summary. Using F_1 female sterility as an indicator of hybrid dysgenesis (HD), we determined the inducing as well as the cytotype-suppressor properties of several P-strains. The data indicate that in P-M hybrid dysgenesis there is not only one type of inducer/suppressor system operative; in some P-strains more than one such system must be present.
Key words. Hybrid dysgenesis; cytotype-suppressor; female sterility; *Drosophila melanogaster*.

Interspecies crosses in *Drosophila melanogaster* lead, in a number of cases, to a syndrome designated hybrid dysgenesis. The best studied examples are the P-M and I-R systems[1,2]. P-M hybrid dysgenesis is characterized by a number of abnormal traits in the F_1 offspring, e.g. female sterility, male recombination and high frequencies of insertion mutations and chromosome rearrangements. These traits are observed only when males from P-strains are crossed to M-strains. It has been found that P-strains carry mobile DNA sequences known as P-elements[3], which are very often of different lengths. The largest completely functional element is 2.9 kb long; smaller elements are formed by internal deletions[4]. Transformation experiments showed that the 2.9 kb P-element can transpose, and also enables in trans the transposition of smaller, non functional P-elements if the latter carry the 31 bp inverted repeats at both ends. It has thus been suggested that the 2.9 kb element codes for a transposase, and perhaps for a suppressor that inactivates P-element activity in crosses of P-females with M-males[4,5].

A comparative analysis of two P-strains, isolated at different times, from the same natural wild-type population in Greece, showed that these two strains exhibit specific differences in their activities. When crossed to several different M-strains, there are differences with respect to the temperature at which they show the highest activity in female sterility and male recombination tests. Moreover, when crossed to a specific strain (*Cy L⁴/Pm*) only one of the two can induce hybrid dysgenesis[6]. These and other results concerning specific deletions and duplications[7] suggested that these two P-strains are different. This observation initiated a more detailed study of the behavior of several P-strains to determine whether 1) P-strains carry identical or different cytotypes and 2) P-strains possess different inducer abilities.

Materials and methods. To characterize the cytotype we crossed inter se 7 different P-strains (6 were isolated from natural wild-type populations, *T-007/Cy, Harwich, π_2, 23.5/Cy L⁴, MR-h12/Cy, 31.1/Cy L⁴;* and one is a laboratory strain, *Cy L⁴/Pm*) and estimated the induced female sterility. To test the inducer ability, the same P-strains were crossed to females of two M-strains (*Canton-S* and *Berlin-K*). The female sterility was measured as described by Yannopoulos[8] and expressed as the percentage of F_1 females that show at least one atrophic (dysgenic) ovary. From each different phenotype of F_1 progeny of all the crosses at least 100 females were examined. All crosses were performed at 29 °C with the exception of crosses involving *23.5/Cy L⁴* and *MR-h12/Cy* males; these crosses were performed at 25 °C, where they have been shown to exhibit the highest activity[6] (and unpublished results). A molecular analysis, using Southern-blot hybridization, showed that all P-strains used, including the *Cy L⁴/Pm* laboratory strain, carry several P-elements, whereas the laboratory wild-type strains, *Canton-S* and *Berlin-K*, are free of P-homologous sequences (Eeken, unpublished; Hatzopoulos and Louis, personal communication). For explanation of gene symbols see Lindsley and Grell[9].

Results and discussion. The results of the crosses to determine the characteristics of the cytotype of the P-strains are shown in table 1. The following P-strains *T-007/Cy, Cy L⁴/Pm, Harwich* and π_2 suppress the activity of *T-007/Cy, Cy L⁴/Pm, Harwich*, π_2 and *31.1/Cy L⁴* but not that of the *23.5/Cy L⁴* and *MR-h12/Cy*.

Table 1. Inter se crosses of different P-strains to determine the cytotype. The females in these crosses are indicated in the first column, whereas the males are in the top line. Induced female sterility is measured and expressed as the percentage of dysgenic females (see text). MR, P-chromosome; Bal, balancer chromosome

		Group 1				Group 2		Group 3
♀♀	♂♂	*T-007/Cy*	*Cy L⁴/Pm*	*Harwich*	π_2	*23.5/Cy L⁴*	*MR-h 12/Cy*	*31.1/Cy L⁴*
Group 1	Genotype of F_1 females							
T-007/Cy	*T-007/MR*	0	1	0	0	94	88	0
	Cy/MR	0	0	0		84	62	0
	T-007/Bal	0	0			0		0
Cy L⁴/Pm	*CyL⁴/MR*	1	0	0.5	1	93	91	2
	Pm/MR	0	0	0	2	87	87	0
	Pm/Bal	0	0			37	62	0
Harwich	*Har/MR*	0.5	2	0	0	92	43	0
	Har/Bal	0	0			0	42	0
π_2	π_2/*MR*	0	1	0	0	24	5	0
	π_2/*Bal*	0	0		0	1	12	0
Group 2								
23.5/Cy L⁴		1	0.5	0	0	0	1	1
MR-h12/Cy		0.5	2	0	0	3	0	0
Group 3								
31.1/Cy L⁴		0.5	4	0	0	0	0	0

Table 2. Crosses of P-strain males to females of *Canton-S* and *Berlin-K* (M-strains) and *Cy L⁴/Pm*, to determine the capacity to induce hybrid dysgenesis sterility. MR, P-chromosome; Bal, Balancer chromosome

♀♀	♂♂	Group 1 *T-007/Cy*	*Cy L⁴/Pm*	*Harwich*	π_2	Group 2 *23.5/Cy L⁴*	*MR-h 12/Cy*	Group 3 *31.1/Cy L⁴*
	Genotype of F_1 females							
Canton-S	*C-S/MR*	100	59	92	70	100	59	100
(C-S)	*C-S/Bal*	4	66			100	38	100
Berlin-K	*BK/MR*	46	51	100	96	91	49	61
(BK)	*BK/Bal*	0.59	73			34	14	96
Cy L⁴/Pm	*Cy L⁴/MR*	0				82	51	0
	Pm/MR	0				78	46	0
	Pm/Bal	0				4	11	0

Note: The *Cy L⁴/Pm* stock described in table 2 (as females) is the original stock used to isolate *31.1* and *23.5* chromosomes[6,11]. This stock was unfortunately lost before the tests with the *Harwich* and π_2 could be performed. The *Cy L⁴/Pm* used in table 1 and in table 2 (as males) was established through the crosses B as described in figure 1 of Yannopoulos et al.[12], and carries the cytoplasm and the first and second chromosomes of the original *Cy L⁴/Pm* stock.

In contrast *23.5/Cy L⁴*, *MR-h12/Cy* and *31.1/Cy L₄* can suppress, nearly completely, the inducing properties of all tested P-strains. From these data it can be concluded that the cytotype of the *T-007/Cy*, *Cy L⁴/Pm*, *Harwich* and π_2 strains (group 1) is different from the cytotype of *23.5/Cy L⁴*, *MR-h12/Cy* and *31.1/Cy L⁴* with respect to the suppressor activity. Moreover the HD-inducing ability of the *23.5* second chromosome is higher than that of the *MR-h12* and *Cy* (from the *MR-h12/Cy* stock) chromosomes, while the *Cy L⁴* chromosome of the *23.5/Cy L⁴* strain can only induce sterility with the *Cy L⁴/Pm* females (group 1). If we compare the crosses between females of the P-strains *23.5/Cy L⁴*, *MR-h12/Cy* and *31.1/Cy L⁴*, with all different P-males, it appears that the cytotype of these strains is identical. However, if we take into account the results of the crosses between males from *23.5/Cy L⁴*, *MR-h12/Cy* and *31.1/Cy L⁴*, these strains seem again to belong to two groups, with respect to their inducing ability: *23.5/Cy L⁴* and *MR-h12/Cy* (group 2) induce sterility with females from group 1, but *31.1/Cy L⁴* (group 3) cannot induce sterility in any of the P-strain females. Based on these results on the suppressor and HD-induction properties of the three groups of P-strains the following conclusion can be drawn. Strains in group 1 carry an inducer (A) that is suppressed by a suppressor-system A′ which is present in all the tested P-strains. The strains of group 2 carry, besides inducer A, most probably also another type of inducer, B, which induces sterility in females from group 1. This type of inducer (B), is however suppressable by the strains *23.5/Cy L⁴* and *MR-h12/Cy* themselves, therefore these two strains must also carry the suppressor-system for B-inducers (B′). The last strain, *31.1/Cy L⁴*, behaves slightly differently. The cytotype of this strain suppresses all P-strains and therefore has suppressor-systems A′ and B′. However, since it cannot induce sterility in females of the first group, it can only carry the A-type inducers. The data indicate clearly the presence of more than one type of inducer/suppressor-system, one of which (A-A′), is in all probability represented by the P-element system. Moreover, another conclusion can be drawn from the data presented. In the crosses of *23.5/Cy L⁴* and *MR-h12/Cy* males to group 1 females the dysgenic activity observed may be due solely to the B-type inducers present in these males, since the A-type inducer activity must be suppressed by the cytotype of the group 1 females. Therefore, it appears legitimate to assume that the observed hybrid dysgenesis is due to B-type inducers and that probably the number of these inducers is higher in the *23.5/Cy L⁴* strain than in *MR-h12/Cy* strain.

We also determined the ability of the P-strains to induce HD in two different M strains. These results are summarized in table 2. All P-strains were crossed to females of two laboratory strains, *Canton-S* and *Berlin-K*, and both strains react as M-strains (hybrid dysgenesis can be induced). However, it is also clear that 1) the inducer capacity of the P-strains varies and 2) *Canton-S* and *Berlin-K* females react differently in spite of the fact that both are completely free of P-elements. It appears as though a suppressor system is present in these M-strains, which cannot be encoded by P-elements. Furthermore, it can be seen that the *Cy L⁴* chromosome of the *23.5/Cy L⁴* and *31.1/Cy L⁴* strains can induce female sterility when crossed to females of the laboratory wild type strains used. The finding that the inducer ability of the *Cy L⁴* chromosome (of the *23.5/Cy L⁴* strain) is suppressed by the cytotype of group 1 strains with the exception of *Cy L⁴/Pm* strain, in contrast to the *23.5* second chromosome (table 1) indicates that this *Cy L⁴* chromosome carries only the A-type inducer while the *23.5* second chromosome must carry both A and B type of inducers.

In summary, it can be stated that the experiments described warrant the conclusion that 1) different types of HD inducers in P-strains can exist, 2) the cytotype of different P-strains may carry more than one suppressor system, 3) the suppressor systems may be determined by factors other than P-elements. Evidence for this is given by the findings of Iwano et al.[10] which suggest that resistance to the P-factor is not necessarily produced by the genetic elements homologous to the P-factor. The apparently different types of HD inducers present in the P-strains studied could possibly represent mutants of the 2.9 kb P-elements. Alternatively we may be faced with the simultaneous presence of a novel non-P-M hybrid dysgenesis system. These possibilities are presently investigated using a molecular approach.

Finally it seems opportune to remark that defining strains of *Drosophila melanogaster* according to their ability to induce female sterility in the F_1 progeny of a given cross to M-females can identify strains only as HD-inducer strains, but gives no information about the possible differences between these strains, and thus HD-inducer strains can only be classified after a more detailed genetic analysis.

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1 Engels, W. R., A. Rev. Genet. *17* (1983) 315.
2 Bregliano, J. C., and Kidwell, M. G., in: Mobile Genetic Elements, Ed. J. A. Shapiro. Academic Press, New York 1983.
3 Bingham, P. M., Kidwell, M. G., and Rubin, G. M., Cell *29* (1982) 995.
4 O'Hare, K., and Rubin, G. M., Cell *34* (1983) 25.
5 Karess, R. E., and Rubin, G. M., Cell *38* (1984) 135.

6 Stamatis, N., Yannopoulos, G., and Pelecanos, M., Genet. Res. *38* (1981) 125.
7 Yannopoulos, G., Stamatis, N., Zacharopoulou, A., and Pelecanos, M., Mutat. Res. *83* (1981) 383.
8 Yannopoulos, G., Genet. Res. *32* (1978) 239.
9 Lindsley, D. L., and Grell, E. H., Carnegie Institute of Washington, Publ. No. 627 (1968).
10 Iwano, M., Kattor, K., Saigo, K., Matsuo, Y., Mukai, T., and Yamazaki, T., Jap. J. Genet. *59*(4) (1984) 403.
11 Yannopoulos, G., and Pelecanos, M., Genet. Res. *29* (1977) 231.
12 Yannopoulos, G., Stamatis, N., and Zacharopoulou, A., Heredity *49*(2) (1982) 235.

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Thalamic stimulation evokes sex-color change and gamete release in a vertebrate hermaphrodite

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Summary. Sperm and egg release and sex-color patterns specific for the male and female phases of reproductive behavior were elicited by electrical stimulation in the thalamus of anesthetized sea bass. Thalamic switching of the sex-role specific motor activities in response to visual signals from the mate is considered an important feature regulating the complex mating activity of these simultaneous hermaphrodites.
Key words. Serranus subligarius; color change; sexual behavior; sperm and egg release; hermaphroditism; thalamus; brain stimulation; hormones and behavior; visual signals; fishes.

Interactions between brain systems for male versus female behavior must occur in hermaphrodites which simultaneously produce both eggs and sperm but normally express only one sex phase at any given time[1,2]. We have studied neural mechanisms for sex-color change and gamete release in one of these unique animals as a means of better understanding such interactions and hence the sensorimotor control of vertebrate reproductive behavior in general.

Serranus subligarius is a small sea bass plentiful along the southern Atlantic and Gulf coasts of the United States. During its daily spawning period, an individual will typically mate as both female and male, with a pair switching roles several times[2-4]. Initially, the fish are noticeably bloated with eggs and exhibit a *dark posterior* (DP) color pattern associated with female behavior (fig. 1B). Following vigorous courtship, the more submissive animal begins to display male-typical behavior and a *banded* (Bd) coloration (fig. 1A). At the height of courting, the male-phase animal is strongly Bd and the individual in the female role retains the DP. As spawning approaches, a normally dark spot on the dorsal fin in the female-phase animal begins to fade. The lightening can also extend down to the side of the body below the spot. The spawning is initiated when the female takes a head-up posture, quivers and flashes a *reverse V* (RV) color pattern that is the complement of the Bd (fig. 1C). The pair then dart toward the surface and perform the spawning act or snap with the 'female' curved in front of the 'male'.

The sex-color patterns of *S. subligarius* are useful for the analysis of neural systems controlling rapid sex change since they are indicators of the sex phases and can be studied in anesthetized-immobilized preparations suitable for neurophysiology. In this project, we mapped brain areas from which the three prominent sex-color patterns of *S. subligarius* (fig. 1 A–C) as well as gamete release can be evoked by electrical stimulation. Experimental procedures were adapted from earlier studies in freshwater fishes[5,6].

Color patterns and gamete release triggered by thalamic stimulation

Site	Fish No.	Evoked response thresholds Bd	DP	RV	SR	ER
1	8	50 μA	—	—	—	—
2	15	60 μA	—	(60 μA)	—	—
3	3	100 μA	—	—	—	—
4	16	—	80 μA	—	—	80 μA
5	7	—	50 μA	(50 μA)	20–50 μA	—
6	15	50 μA	—	—	—	—
7	14	50 μA	—	—	—	—
8	12	80 μA	—	20 μA; (50 μA)*	50–100μA	—

Bd, banding; DP, dark posterior; ER, egg release; RV, reverse V; SR, sperm release; ( ), after-response at termination of stimulation: *weak responses were evoked during 20 μA stimulation while strong after-response occurred following some of the tests of 50 μA.

Fish were captured during July and August using SCUBA at St. Andrews State Park, Florida. With the exception of one animal (see below), they were transported to the Gulf Coast Research Laboratory, Ocean Springs, MS, and maintained in stock tanks. Most fish were tested within 1 week of capture while still spermiated but not before they stopped ovulating which occurred after 1 or 2 days. Our data on female color patterns and egg release are thus limited. Fish were anesthetized in 2% urethane and then placed in a surgical holder and perfused through the mouth with seawater (fig. 1D). Evoked color changes were videotaped for later analysis and gamete release was monitored by placing a small container of seawater under the fish's abdomen (fig. 1E). Electrodes were either tapered stainless steel insect pins or commercially available microelectrodes. Stimulation was provided by a Grass S88 stimulator and PSIU-4 constant current converter and consisted of 50 Hz, 1-ms square-waves with currents up to 100 μA. Electrodes were lowered from the dorsal surface of the exposed brain with current set at 100 μA. When a positive response occurred, the current was reduced and the electrode moved up and down to find the most sensitive area. Evoked responses were repeatable (at least 5 × consecutively) with similar latencies (approximately 10–30 s). Areas of interest were marked by iron deposition for identification using the Prussian blue reaction with a neutral red counterstain.

An attempt was made to sample all major areas of the brain. Sex-related color changes were elicited on 48 electrode tracks in 25 animals and 15 sites in 10 fish were identified. Many other tracks were negative. This paper deals specificially with 8 sites clustered in the thalamus, the only area from which all three color patterns were evoked (fig. 2). Other sites positive for 1 or 2 of the responses were located in the area ventralis telencephali pars supracommissuralis, the preoptic area and adjacent optic nerves, the optic nerve and adjacent pretectum-thalamus, the torus semicircularis and ventral tegmentum of the midbrain and medial reticular formation of the medulla. The latter results have been described elsewhere[2,7].

As mentioned, each of the three color patterns was evoked by thalamic stimulation; in most cases more than one response was