SHORT PAPER

Hybrid dysgenesis in natural populations of *Drosophila melanogaster* in Japan. I. Complete absence of the *P* factor in an island population¹⁾

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There is growing evidence that the *P* factor is a moderately repetitive, dispersed and apparently movable element, whose multiple copies are carried by many *Drosophila melanogaster* genomes (Engels 1981). The *P* factor is defined by its ability to bring about, under certain well specified conditions, a state called hybrid dysgenesis (Kidwell *et al.* 1977). One of essential features shared by abnormal germ line behavior is that dysgenesis occurs primarily in just one of the two reciprocal cross hybrids, although these two hybrids are chromosomally identical. Based on the asymmetry we can categorize strains as either maternally (*M*) or paternally (*P*) contributing to any given dysgenic trait.

The causative agent for hybrid dysgenesis, the *P* factor, appears to present at multiple locations in the genomes of *P* strains and be absent from *M* strains. Furthermore, the determination of susceptibility or immunity to the sterilizing action of the *P* factor, referred to as the *M* or *P* cytotype, respectively, involves chromosomal factors and limited cytoplasmic transmission (Engels 1979). The simultaneous presence of the *P* factor and *M* cytotype in the germ line is both necessary and sufficient for hybrid dysgenesis to occur.

When dysgenic hybrids from the cross *M* female $\times$ *P* male are raised at restrictive temperature ($>27^{\circ}\text{C}$), they are frequently unable to produce gametes. This type of sterility, called gonadal dysgenesis is observed as failure to produce eggs, and appears when dysgenic daughters are subjected to restrictive temperature condition during late embryonic and early larval stages (Engels and Preston 1979; Schaefer *et al.* 1979).

Sterility is a convenient indicator of hybrid dysgenesis and this has been used for detailed studies of the genetic mechanism involved.

Previous studies (Picard *et al.* 1978; Kidwell *et al.* 1977; Kidwell 1979, 1982;

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Engels and Preston 1980) have shown that strains recently taken from nature tend to be *P*. In contrast, long-established laboratory strains are almost always of the *M* type. However, there are some exceptions. Kidwell (1982) reports that *M* populations apparently still exist in several locations in the USSR and Australia.

Two models have been advanced to explain the evolution of the present-day distribution of laboratory strains and natural populations. The stochastic loss hypothesis of the *P* factor proposed by Engels (1981) is one of the models. A quite different explanation, the recent invasion hypothesis, has been proposed by Kidwell (1979, 1982). Little if any evidence has previously been available to aid in distinguishing between these two types of hypothesis.

In this view, findings concerning the geographical distribution of strains in the *P-M* system especially from the area not studied yet should be important. So far as the *P-M* system is concerned, wild populations around Japan have not been reported.

In this paper, we describe briefly a particular wild population of *Drosophila melanogaster* from the Bonin Islands of Japan with respect to the components of the *P-M* system of hybrid dysgenesis. As a measure of dysgenesis we use female sterility called gonadal dysgenesis. In the present experiment our primary interest is to reveal the distribution of the two components, the *P* factor and cytotype among members of the population.

The present study shows that the *P* factor is completely absent in this island population, and cytotype is mostly resistant to the *P* factor. Therefore, this population appears to be unique in that it lacks the *P* factor, but resistant to hybrid dysgenesis, unlike an ordinary laboratory stock which lacks the *P* but susceptible to the dysgenesis. Another system of hybrid dysgenesis, the *I-R* system has not been considered in this experiment.

Wild population samples: A large population of *Drosophila simulans*, *D. melanogaster*, and other species of *Drosophila* inhabit in Chichijima Island, one of the biggest among the Bonin Islands, located in the Pacific Ocean about 1,000 km southeast of Tokyo, at about the same latitude as Florida. The present human population of Chichijima is about 1300. Some genetical informations such as Ne and protein polymorphism can be found in Yamaguchi *et al.* (1980).

In June 1979 about 1000 females of *Drosophila melanogaster* were collected from open traps containing banana and live yeast. Immediately after the capture, they were transferred to a regular size population cage with 15 cups containing a conventional cornmeal media and was maintained at 19°C in our laboratory for about two years. This population cage can support approximately three to four thousand flies.

Male flies from the cage were used directly for the crosses A and B*, des-

cribed below. Females already mated in the cage were placed singly in vials, and two daughters from each vial were used for the cross A*. Some additional daughters were obtained for the cross B. Test matings were carried out at 29°C which is restrictive temperature for the gonadal dysgenic sterility. We dissected every sterile female to distinguish the background sterility from gonadal dysgenesis.

Strains used: A laboratory stock, Canton-S was used as the standard *M* strain. A wild strain, called Harwich, was the standard *P* strain (Kidwell 1979).

Method of monitoring strains in the P-M system: In order to test the presence of the *P* factor in an unknown strain U by sterility tests, we made the following reciprocal crosses to obtain daughters:

- (A) Canton-S ♀♀ × U ♂♂
 (B) U ♀ × Canton-S ♂♂.

And the cytotype of an unknown strain U can be determined by the following crosses:

- (A)* U ♀ × Harwich ♂♂
 (B)* Harwich ♀♀ × U ♂♂.

Sterility tests: Test matings were carried out at 29°C for 7 days, and all progeny emerging by the 11th day were transferred to fresh mating vials kept at 25°C. After four additional days, each female daughter to be tested was placed in a cell of an egg laying box with 60 cells. This container is prepared in a similar way to that of Engels and Preston (1979, 1980). After three days, each female was scored as either sterile, if no egg found, or fertile if one or more eggs observed. Among sterile, only those females having two rudimentary ovaries were counted as gonadal dysgenesis, (see Engels and Preston 1980). From a standard dysgenic cross (Canton-S ♀♀ × Harwich ♂♂) following the present protocol, close to 100% sterility was observed with the background sterility being negligibly small.

Test for the presence of the P factor: A total of 273 males from the cage population were tested for the presence of the *P* factor using the cross A for each of which we tested 15 daughters for the gonadal sterility. This test has revealed that the *P* factor is completely absent in this particular population. And the average sterility was about 0.46%. Furthermore, dissections of those sterile females showed that none were of the gonadal dysgenic type. Thus they were due to the background sterility. For certainty of the background sterility, the cross B was performed for two female offsprings from each of 69 wild females sampled from the cage population. Each female was mated

Table 1. *Distribution of the numbers of sterile daughters among 15 daughters tested from each of 440 females examined for cytotype*

No. of sterile daughters	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
No. of females derived from the cage	232	29	12	11	14	20	12	15	14	14	5	13	10	14	10	15	440

Average sterility (%) 23.0

Fig. 1. Distribution of the *P* cytotype among 440 females whose cytotype are the result of matings that occurred in the population cage. Fifteen daughters are tested for sterility from each female.

to Canton-S males, and 15 daughters each were tested for sterility. The frequency of sterility was very low (0.24%) and close to that of the reciprocal cross.

Determination of cytotype: From each of the 226 females sampled from the cage population, two virgin female offsprings were collected and mated individually to Harwich males at 29°C. Then, approximately 15 daughters were tested for sterility from each of the 440 successful A* mating thus initiated. The cytotypes under consideration come from matings that have occurred in the population cage. Table 1 and Figure 1 show the number of females producing various degree of sterile daughters. The results imply that the *P* cytotype is predominant in this population. The vast majority produced few or no sterile daughters. As shown in Table 1, the average value of the steri-

lity among about 6600 daughters (226 lines $\times$ 2 sib duplications $\times$ 15 hybrid daughters) was 23.0 percent. It should be also mentioned that the *M* cytotype may not be completely absent. There were 15 females with their daughters being all sterile (15/15). However, only two came from a single female immediately derived from the cage and the rest of them were independent.

From the cross B* there was no gonadal dysgenesis. Of 3 sterile females (0.34% of the total tested) none was gonadal dysgenic. As mentioned above, every tested male appears to lack the potential for the dysgenic sterility when crossed to *M* females. We may, therefore, conclude that most flies in this population are neutral with respect to gonadal dysgenesis.

Discussions: The mechanism causing this apparent neutrality remains unknown. However we are tempted to speculate that the *P* cytotype observed here is due to the *Q* factor of Kidwell (1979). The *Q* factor appears to be in some ways an incomplete, or defective, form of the *P*, (Engels and Preston 1981; Kidwell 1982). It may be defective in manifesting gonadal dysgenesis, but able to convert cytoplasm to the *P* type which is resistant to hybrid dysgenesis (Simmons *et al.* 1980; Engels and Preston 1981; Kidwell 1981; Kidwell *et al.* 1981). Taking their results into consideration, intermediate sterility in Figure 1 suggests that the population is polymorphic for the *Q* factor (Engels and Preston 1980). However, if the degree of the resistance were slightly weaker due to its defectiveness than that caused by the *P* factor, there would be still two possibilities to cause the intermediate sterility. One might be that, though every individual carries sufficient amount of the cytoplasmic factor caused by the *Q*, the effect is still too weak to produce a complete resistance to hybrid dysgenesis. The other possibility would be that, because of the assumed defectiveness of the *Q*, the amount of the cytoplasmic factor is not sufficient to be distributed to all individuals and therefore some individuals lacking the factor may be only partially resistant. In the latter case, we are implicitly implying that the population is polymorphic in the cytoplasmic factor assumed to be produced by the *Q*.

Presently we are attempting to analyse more detail structure of the cage population with respect to the degree of resistance to hybrid dysgenesis. In doing this, we are isolating a large number of iso-female lines and selecting lines consistently showing susceptible cytotype or resistant *P* cytotype. The results will be reported elsewhere.

According to Kidwell (1982) the *Q* factor may be distributed around the world. The relative frequencies of the two factor, *P* and *Q*, however, vary in different subpopulations. Our preliminary study have found that the *P* factor seems to exist in other parts of Japan. For instance, an analysis of a Sapporo population has revealed that the *P* factor is indeed maintained in this area, but at low frequencies. It also appears that this area maintains the *Q* factor

at relatively high frequencies. Therefore, large fractions of cytoplasms are resistant to the *P* factor. However, to reach a definite conclusion, it requires more data.

The origin of the apparent neutrality found in the cage population derived from Chichijima Island remains unanswered. We may also ask why the island population entirely has the *Q* rather than the *P* which is more commonly found in North American populations. A naive interpretation can be that the *P* is more disadvantageous than the *Q* in areas with temperature high enough to allow the manifestation of hybrid dysgenesis, if such a circumstance should arise. This is, of course, only speculation, but can be easily tested. In fact we are carrying out this survey using flies collected from a number of locations extending from south to north.

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