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Evolution

Cytotype polymorphism of the *P–M* system in two wild populations of *Drosophila melanogaster*

(genetic variability/mutational events/hybrid dysgenesis/population evolution)

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ABSTRACT **In *Drosophila melanogaster* the interactions of the *P–M* system generate germ-line aberrations (e.g., sterility, mutations) found in certain interstrain hybrids. Two wild populations, from France and Tunisia, were examined in order to determine the distribution of the chromosomal *P* factor and the extrachromosomal cytotypes. No *P* factors active for potential *GD* sterility were found in these populations. But search for the *M* cytotype, which causes susceptibility to the *P* factors, and for the *P* cytotype, which causes resistance to the *P* factors, showed that both populations are polymorphic for the cytotypes. Such a polymorphism seems to be stable in the wild, at least over a 2-year period. Mutator activity (measured by generated mutations at the *sn* and *ras* loci) was found to be present. Some possible interactions between cytotype polymorphism, mutator activity, and the structure of *Drosophila* populations are discussed.**

In *Drosophila melanogaster* the interactions of the *P–M* system of "hybrid dysgenesis" has been extensively described (1–4). These interactions result in germ-line aberrations in certain interstrain hybrids and include temperature-sensitivity sterility (*GD* sterility), high frequencies of mutations and chromosome rearrangements, male recombination, and transmission ratio distortion. They can be recognized by their reciprocal cross effect. That is, strains can be classified as either *P* or *M* such that hybrids from crosses of the form *M*♀ × *P*♂ show dysgenic traits that are reduced or absent in the reciprocal hybrids. A third category of so-called *Q* strain, neutral at least for *GD* sterility, has also been described that gives nondysgenic progeny whatever way the crosses with *P* or *M* strains (5, 6) are made.

The dysgenic traits appear to result from the interaction of two genetic components: a chromosomal component consisting of polygenic factors (*P* factors) linked to one or more of the major chromosomes of paternally contributing (*P*) strains and the extrachromosomal component, referred to as the *M* cytotype, transmitted maternally from the *M* strain (5). The dispersed and probably mobile (4, 7) *P* factors, which are present in the *P* strains and absent or inefficient in the *M* and *Q* strains, do not normally cause dysgenic traits within homogeneous *P* or *Q* strains because of resistance conferred by the extrachromosomal condition referred to as the *P* cytotype.

The inheritance of cytotype has characteristics of both classical cytoplasmic and Mendelian heredity. Cytotype is primarily controlled by chromosomal determinants, simultaneously present in many genomic locations, but the maternal extrachromosomal condition can have, under some circumstances, an important short-term effect (5, 8). The occurrence of a cytotype switch appears to be a stochastic event, the probability of which is determined jointly by an individual's own genotype and by its mother's cytotype. Each of the three major chromosomes has been shown to possess the ability to switch the cytotype (5), but their functional relationships with the *P* factors are still the subject of hypotheses (9, 10).

The distribution of *P*, *M*, and *Q* types in natural populations has been studied in North America, Europe, and Africa (4, 11). High frequencies of *P* strains are a characteristic of populations from the United States, in which *P* factors were found to be common, with chromosomes being polymorphic for these factors, whereas *M* cytotype is absent, or present at only very low frequencies (12, 13). On the contrary, the frequency of *P* strains appears markedly lower in central Africa and practically null in current European and Mediterranean populations, in which *Q* strains are the most frequent but *M* strains fairly common. Because the level of activity of the *M* cytotype in these latter populations varies remarkably, the existence of a polymorphism of the *M* and *P* cytotypes was assumed (11). The incidence of this polymorphism appears particularly important for the knowledge of the population genetics of the *P–M* system and of its evolution in nature. The test of the polymorphism hypothesis represents the subject of this communication.

MATERIALS AND METHODS

Two Mediterranean wild populations sampled in 1981 (each at least 50 individuals) in Tautavel village, Pyrénées-Orientales, France, and in Nasr'Allah, an oasis in South Tunisia, and which present *M* cytotype (11) were used. They were kept in standard laboratory conditions by mass culture of about 2,000 individuals for Tautavel village and by 54 isofemale lines directly started from the wild-caught females for Nasr'Allah. Each isofemale line had a population size of about 300 individuals. Both populations were analyzed during their first five generations after their capture. Single individuals, 115 males and 103 females randomly chosen from the Tautavel village population and 432 males and 108 females (8 males and 2 females taken in each isofemale line) from the Nasr'Allah population, were characterized at 28.5°C by using the *GD* sterility criterion (8) in the following way: cross A = 3 Canton S (*M*) ♀♀ × 1 ♂ of population under test; cross A* = 1 ♀ of population under test × 3 Harwich (*P*) ♂♂. Dissection of 25 F_1 females 3–5 days after emergence allowed us to determine the presence of unilateral (S1 type) or bilateral (S0 type) atrophic gonads and to estimate the frequency of *GD* sterility by the percentage of individual dysgenic ovaries (% *GD* = % S0 + 1/2 % S1). Cross A provided a measure of the activity of *P* factors in males, and *P* strains are not expected to produce more than trivial levels of *GD* sterility in cross A*. Cross A* distinguishes between *M* cytotype (high frequency of *GD* sterility) and *P* cytotype (only trivial levels of *GD* sterility). As a control 74 individual crosses (1 ♀ Canton S × 1 ♂ Harwich) were similarly performed on reference strains to determine the

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residual variability of *GD* sterility in our experimental conditions.

The existence of mutator activity in the Nasr'Allah population was tested by the observation of the induction of X-linked recessive mutations in the F_1 sons from a cross between Nasr'Allah males and either Canton S or Nasr'Allah females. The competence of induction of visible mutants was determined by the specific locus method (14), using a yellow, singed, raspberry, vermilion (y^2 sn^3 *ras* *v*) strain. Fifty newly emerged males from one Nasr'Allah line, selected randomly among the lines presenting a *P* cytotype, were crossed in mass, one half with Canton S females and the other half with Nasr'Allah females, taken in one line selected randomly among the lines presenting an *M* cytotype. The first cross reveals the mutator potentiality of some males of the natural populations. With the second cross one can see if this mutator potentiality may also be expressed in the population itself. In both cases, 100 F_1 males were then crossed in groups of ten, in ten independent replicates, to y^2 sn^3 *ras* *v* females and their daughters were scored for the *y*, *sn*, *ras*, and *v* phenotypes.

RESULTS

The results of the three experiments are given in Table 1 and Fig. 1. In both populations *P* factors active for *GD* sterility were not revealed, confirming previous study of *Drosophila* populations collected in these areas (11). On the contrary, both populations show the existence of *M* cytotype with an overall proportion of gonadal dysgenesis about 22.1% for Tautavel village and 72.7% for Nasr'Allah. Because two females were taken from each of the 54 Nasr'Allah lines to be tested for cytotype, a nonparametric measure of correlation was made to test the similarity between pairs. This similarity was high (ρ of Spearman $= 0.56$, $P < 0.001$) showing that cytotypes were well characterized. As mentioned earlier in this paper, intermediate and different levels of *GD* sterility raise the problem of the existence of a cytotype polymorphism. Fig. 1 clearly shows that such a polymorphism does exist in both populations. As a matter of fact, if the population were monomorphic, there would be a constant probability of sterility, yielding a binomial distribution. Instead of which, the frequency distributions of *GD* sterility among the offspring of individual females from both populations are found to be strongly bimodal. These results show the presence, in natural populations, of the two fairly distinct cytotypes. Moreover, these distributions strongly differ from the distribution obtained from the Canton S strain, homogeneous for *M* cytotype, in which only one mode has been found. It is interesting to note that, in nature, a clear-cut difference appears between *P* and *M* cytotypes, recalling the "all-or-nothing" cytotype switch observed in experimental populations (5).

In our case, looking at the bimodal distribution of sterility frequency (Fig. 1) and selecting a cutoff point at 10% to classify the cytotypes, the cytotype polymorphism observed in the wild shows different values, with 27.4% of individuals of *M* cytotype in Tautavel village and 88.8% of *M* cytotype individuals in the Nasr'Allah population. It is worth noticing that these values correspond to the level of the overall mean activity of the *M* cytotypes as routinely measured by testing 30 females randomly sampled in the base population, namely 37% in Tautavel village and 80% in Nasr'Allah (11). Thus a cytotype polymorphism implicates an intermediate level of overall mean activity of the *M*

Table 1. Mean percent of F_1 female *GD* sterility in the tests for *P* factor activity (cross A) and *M* cytotype (cross A*) of Tautavel village and Nasr'Allah populations

Strain tested	% gonadal type							
	Cross A (Canton S ♀♀ × ♂♂ tested)				Cross A* (♀♀ tested × Harwich ♂♂)			
	S2	S1	S0	*GD*	S2	S1	S0	*GD*
Tautavel village	99.7	0.2	0.1	0.2	74.8	6.3	18.9	22.1
Nasr'Allah	99.8	0.1	0.1	0.2	23.3	8.0	68.7	72.7
Canton S controls	—	—	—	—	4.7	11.5	83.8	89.6

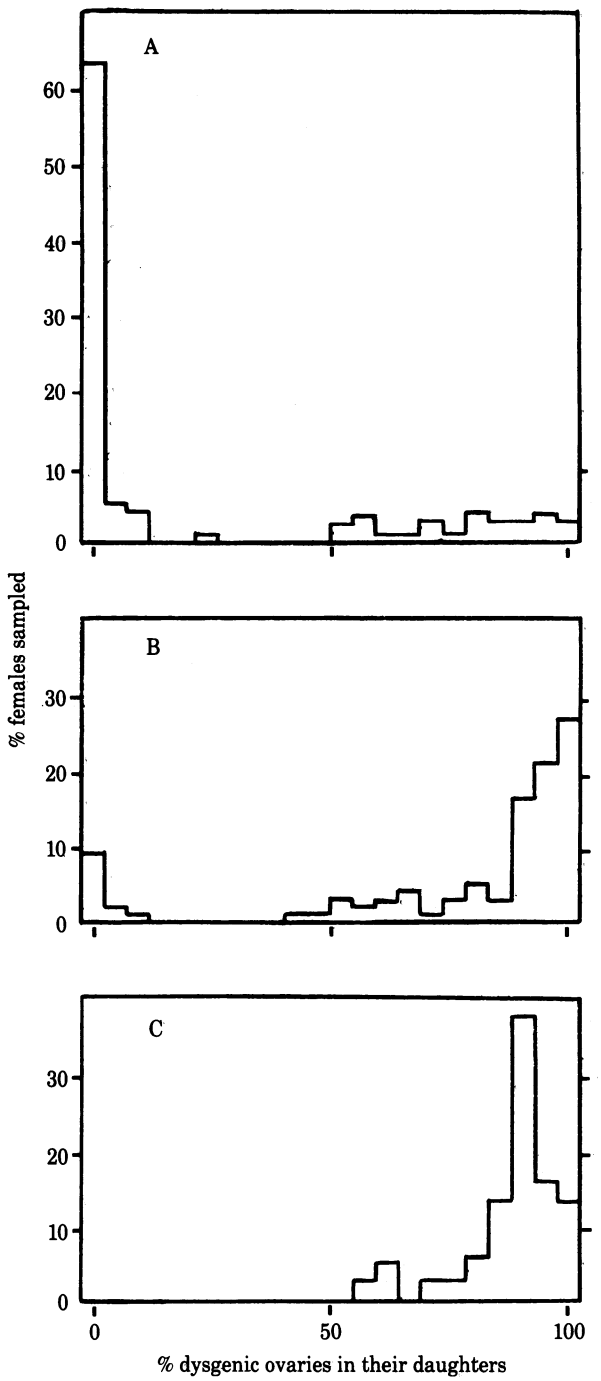

FIG. 1. Distribution of sterility frequency among the progeny of females sampled in Tautavel village (103 females) (*A*) or Nasr'Allah (108 females) (*B*) and from the Canton S strain (74 females) (*C*). *GD* sterility was estimated by the mean percent dysgenic ovaries of 25 daughters from individual crosses with Harwich males.

cytotype, the opposite of which is not necessarily true. Nevertheless, the observation of an intermediate level of overall mean activity of the *M* cytotype in a wild population suggests the existence of cytotype polymorphism, particularly in other recently sampled (1981–1982) populations from Portugal, Spain, France, Algeria, Tunisia, Egypt, and India. Moreover, in Nasr'Allah this polymorphism seems to be stable over a 2-year period, populations collected between October 1979 and October 1981 revealing the same level of mean activity of the *M* cytotype (11).

In the experiment of mutability no mutants were found at the *y* and *v* loci in either cross, an observation that corresponds with the low induced rates of mutation found at these loci in comparable experiments by other workers (14). The measure of the average mutator activity by mutations induced at the *sn* and *ras* loci appeared not to be different for males crossed either with Canton S or with Nasr'Allah females. The frequencies were estimated at 1.1 mutation per 10^4 chromosomes scored (4/36,009) for the *sn* locus and 0.7 per 10^4 for the *ras* locus (2/27,306). These values, which are the mean overall mutator activities of the tested males, are of course lower than the 7.7×10^{-4} (*sn* locus) and 3.4×10^{-4} (*ras* locus) values obtained for the more powerful *MR* chromosome isolated from one individual in another wild population (14), and frequencies exceeding these values by approximately 50-fold were also reported (15). Nevertheless, the values we observed are higher than the spontaneous frequency of mutation in laboratory populations, 2×10^{-6} at the *sn* locus and 4×10^{-6} at the *ras* locus (14). Moreover, the *sn* mutants were viable, whereas the two *ras* mutants were not. These results show that mutator elements are present and can generate some mutational events in the Nasr'Allah population.

DISCUSSION

Because the *P–M* system is made of two components, each of them needs to be investigated in order to understand the role and the evolution of this system. Studies in natural U.S. populations have led to the observation of an important polymorphism for chromosomal *P* factor but principally a monomorphism for the *P* cytotype, the *M* cytotype being absent or rare (12, 13). Observations at the molecular level have shown the presence of a family of dispersed repeated DNA segments (called *P* elements) in *P* strains but not in most *M* strains (15). This family is highly heterogeneous in size and structure and its elements might be functionally different. It has been suggested that *P* strains harbor *P* elements that appear to have functional potential for sterility, mutator activity, and other functions (16). In the same way, *Q* strains would possess *Q* elements that may be defective or silent *P* elements and appear to lack sterility potentiality while retaining mutator activity and other functions (4, 16, 17). Thus polymorphism occurring in natural populations for the *P* factor could be explained by qualitative and quantitative variations of these elements in different populations (16, 18).

Our finding of polymorphism for *M* and *P* cytotypes in Mediterranean countries with the simultaneous absence of *P* factor having a sterility potentiality raises the question of the nature of the *M* and *Q* individuals encountered in these populations. If *M* individuals can probably be interpreted as having *M* cytotype and lacking *P* or *Q* elements, *Q* individuals might have either a *P* cytotype and *Q* elements or *P* cytotype and no elements. The question of the existence of elements in these populations is of interest for our knowledge of the evolution of the *P–M* system. Actually it is plausible that such elements devoid of *GD* sterility functions do exist in these cytotype polymorphic populations. In the Nasr'Allah population the observation of mutator activity militates in favor of the existence of such elements. It is worth noting that both Nasr'Allah and Canton S strains have been characterized as being of the *I* type in the *I–R* system of hybrid dysgenesis (ref. 19 and unpublished observation). Because *I–R* interactions do not generate mutations in crosses of the same type (4), the present *sn*- and *ras*-induced mutations may be attributed to the *P–M* interactions. Thus the Nasr'Allah population, polymorphic for the cytotypes and devoid of *P* factors with potential *GD* sterility, seems nevertheless to possess elements with mutational activity.

In this last case one can expect the manifestation of the different mutational events produced by the *P–M* system and the genesis of genetic variability in wild populations, as has been suggested for similar systems in which the genesis of mutations is thought to occur in the wild and not only during hybridization between wild and laboratory strains (18, 20–23).

Finally, in these populations the (re)appearance of potential *GD* sterility, which could result from the introduction or the multiplication of active *P* elements or, more excitingly, from the transformation of *Q* elements into *P* elements, is clearly an important question for the analysis of genetic polymorphism in natural populations.

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