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Temporal stability of P–M cytotype polymorphism in a natural population of *Drosophila melanogaster*

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Summary

The P–M system of hybrid dysgenesis in *Drosophila melanogaster* is a syndrome of genetic abnormalities which appears in the progeny of crosses between strains different with regard to their possession of 'P' transposable elements. Cytotype is an extrachromosomal property which regulates the mobility of the P element. We report here data showing that a cytotype polymorphism previously observed in a natural population from North-Africa is stable over a period of 5 years. A potentially high rate of mutation is associated with this cytotype polymorphism. Explanations of the appearance of a cytotype polymorphism are proposed and the consequences for the genetic load induced by transposable elements are discussed.

Introduction

In natural populations of *Drosophila melanogaster* there is an important polymorphism for both the number of copies of transposable elements and the chromosomal distribution of these copies (Montgomery & Langley, 1983; Biémont, 1986; Ronsseray & Anxolabéhère, 1986; Leigh-Brown & Moss, 1986; Ajioka & Eanes, 1986). The P and I transposable element families are responsible for the phenomenon of 'hybrid dysgenesis' through the P–M and I–R systems (for review see Bregliano & Kidwell 1983; Engels, 1983). Characteristics of the P–M system of hybrid dysgenesis may also show an important polymorphism in natural populations. Engels & Preston (1980) and Kidwell (1980) have shown that American populations present a polymorphism for 'P activity' which reflects the ability of the P elements to produce transposase. Anxolabéhère *et al.* (1982*b*) have shown the existence of a polymorphism for 'cytotype', an extrachromosomal property which regulates the mobility of the P elements. A so-called 'M' cytotype allows the P elements to transpose whilst a 'P' cytotype represses this transposition (Engels, 1981). Cytotype is partially maternally inherited but also determined by the P elements carried on the chromosomes (Engels, 1979). The existence of a cytotype polymorphism is of particular interest because the co-occurrence of the two kinds of cytotype (M and P) in a given population may result in the production of new mutations due to P element insertions (Anxolabéhère *et al.* 1982*b*). There is strong evidence that P elements have only relatively recently appeared in natural populations of *D. melanogaster* (Kidwell, 1983) and will probably be present in large numbers in all populations in the near future (Anxolabéhère *et al.* 1985). It is therefore possible that cytotype polymorphism will be transient, corresponding to a given moment in the P element invasion of the population. This is supported by results from experimental populations in which artificially induced cytotype polymorphisms naturally evolve towards the P state (Kidwell *et al.* 1981; Anxolabéhère *et al.* 1986).

In this article we measure the temporal stability of cytotype polymorphism and of the ability to produce a high rate of mutations in a natural population of the '*M*'' type (a population showing P elements, but devoid of P activity, and in which some individuals present an M cytotype). The results clearly show the stability of these two parameters over five years, and suggest that a high mutation rate induced by transposable elements may be a long-term phenomenon.

Materials and Methods

(i) *Wild population samples*

The wild population from Nasr'Allah (an oasis in south Tunisia) originally sampled in 1981, which was previously used by Anxolabéhère *et al.* (1982*b*), was sampled again in 1983, 1984, 1985 and 1986 (40, 61, 81 and 53 isofemale lines respectively). Each female was

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taken from a different fruit, at least 50 meters apart. Given the density of the original population, we can consider that these lines are genuinely independent. All lines were maintained under mass-culture at 18 °C in non-overlapping generations.

(ii) *Laboratory stocks*

Harwich (*P*): a strong P reference strain descended from two females collected in Harwich (Massachusetts) in 1967.

Canton-S (*M*): a strong M reference strain, collected in Ohio in 1930.

y, *sn*, *ras*, *v* (*M*): a multiply marked X-chromosome line

(iii) *Cytotype and P activity determination*

From each line under test, three virgin females were crossed with Harwich males at 29 °C, a temperature which is restrictive for gonadal sterility specific to the P–M system – This 'G–D' sterility is known to reflect the intensity of the dysgenesis (Kidwell *et al.* 1977) and was quantified as follows: 50 F1 females were left to age three to five days after eclosion before dissection. Their ovaries were then examined. The frequency of dysgenesic ovaries (G–D sterility) was calculated as the number of atrophic ovaries divided by the total number of ovaries examined. High frequencies of sterility produced in this cross are indicative of M cytotype and low frequencies (under 5%) are indicative of P cytotype. P activity of males was determined by crossing three males from each line under test with three Canton-S virgin females and the frequency of G–D sterility in the F1 females was determined as described above. A P type line produces more than 5% of G–D sterility.

(iv) *Mutator activity determination*

Following previously established procedure (Anxolabéhère *et al.* 1982*b*), mutator activity determination in the Nasr'Allah population collected in 1985 was tested by the observation of *X*-linked recessive mutations in the F1 sons from a cross between Nasr'Allah males and Nasr'Allah females. Twenty males from a Nasr'Allah line, selected randomly among the lines presenting P cytotype, were mass-crossed with twenty virgin females taken from a Nasr'Allah line selected randomly among the lines presenting M cytotype. Three replicates were made with three lines presenting P cytotype and three presenting M cytotype. In each cross, 100 F1 males were crossed in groups of ten, in independent replicates, to *y sn ras v* females. The female offspring of these crosses were then scored for the *y*, *sn*, *ras* and *v* phenotypes.

3. Results

(i) *Maintenance of a cytotype polymorphism in the Nasr'Allah population*

Each year the isofemale lines were tested less than five generations after capture, for both P activity and cytotype state. No line showed significant P activity. However, cytotype as measured by GD sterility presents various levels (0–100%) between lines. In order to compare the cytotype distributions between the different years of capture, data were classed by steps of 5%. The frequency of each class is presented in Fig. 1. As previously found in the sample collected in 1981 (Anxolabéhère *et al* 1982*b*), all of the samples collected present a cytotype polymorphism, that is the co-occurrence, in a given sample, of lines presenting an M cytotype and others presenting a P or intermediate cytotype. The respective proportions of these classes do not differ significantly between years. If 25% and 75% of GD sterility are taken as threshold values, the samples are not different ($\chi^2 = 10{\cdot}8$, 8 D.F.: $P > 0{\cdot}20$). In general the mean values of cytotype show very little inter-year difference (see Fig. 1). The Nasr'Allah population seems to be stabilized with about 10%, 70% and 20% of individuals showing cytotype levels under 25%, over 75% and between these values respectively.

(ii) *Cytotype of individuals from isofemales lines presenting intermediate cytotype.*

Fig. 2 shows cytotype distributions of three isofemale lines taken from the 1984 sample five generations after their capture, all of which present an intermediate cytotype level. From each of these lines, 20 virgin females were individually crossed with Harwich males. The percentage of GD sterility in their F1 gives a measure of the cytotype of the females from these lines. A clear-cut difference appears between P and M cytotypes, demonstrating the segregation of genetic determinants of cytotype in isofemale lines presenting an intermediate cytotype.

(iii) *Mutator activity*

The co-occurrence of M and P cytotype in the Nasr'Allah population results in dysgenic individuals with a high rate of mutation in the germline of F1 females. This activity was measured in the 1985 sample as outlined in the Materials and methods. The observed mutation rate was $0{\cdot}7 \times 10^{-4}$ (1/12832) for the *sn* locus and $3{\cdot}9 \times 10^{-4}$ (5/12832) for the *ras* locus. This is of the same order of magnitude as the frequencies observed in the 1981 sample ($1{\cdot}1 \times 10^{-4}$ for the *sn* locus and $0{\cdot}7 \times 10^{-4}$ for *ras* locus) and much greater than the spontaneous frequency of mutation observed in laboratory populations (2×10^{-6} for *sn* and 4×10^{-6} for *ras* (Green, 1977). The high rate of

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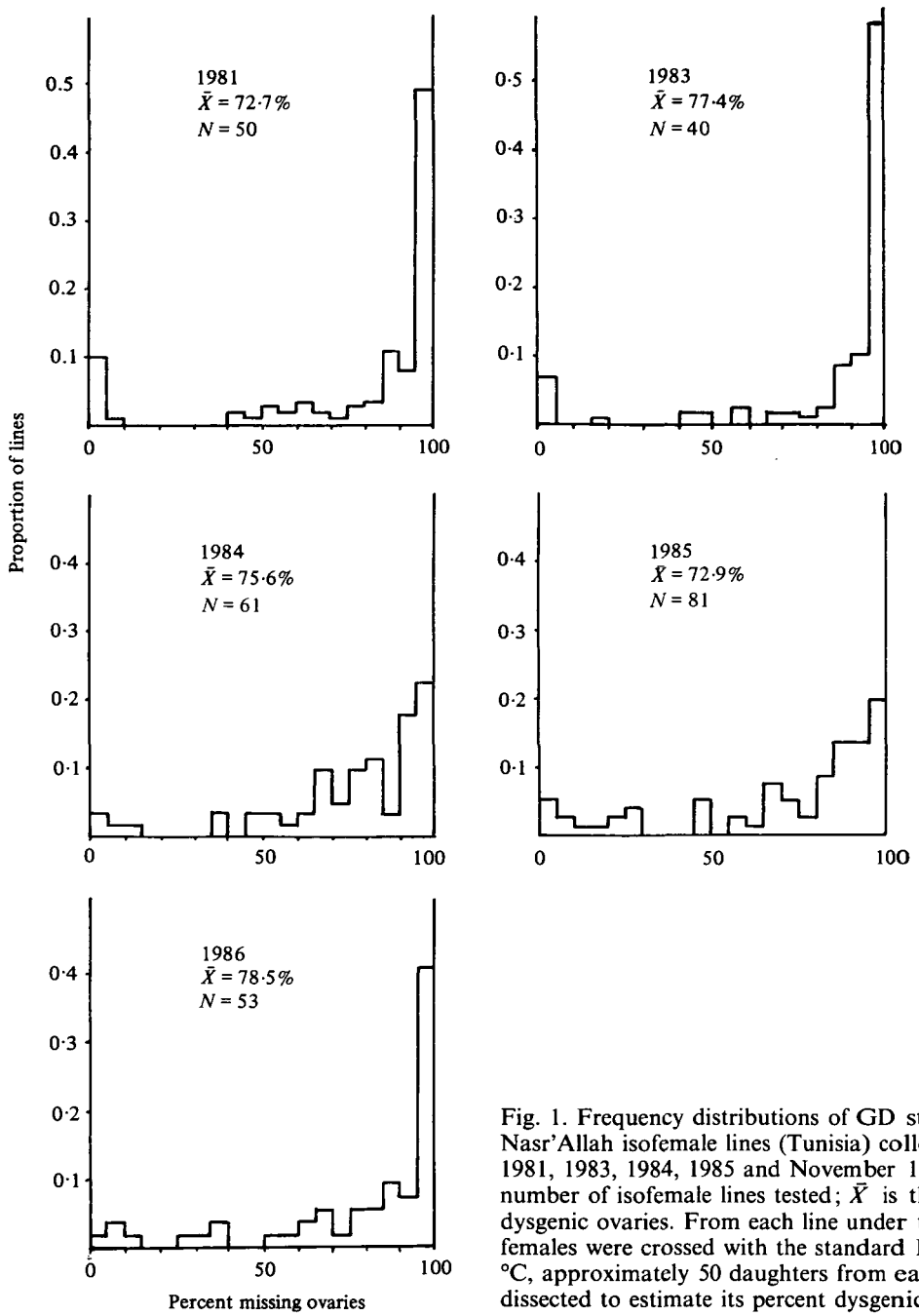

Fig. 1. Frequency distributions of GD sterility of Nasr'Allah isofemale lines (Tunisia) collected in October 1981, 1983, 1984, 1985 and November 1986. N is the number of isofemale lines tested; $\bar{X}$ is the mean percent dysgenic ovaries. From each line under text, three virgin females were crossed with the standard P strain at 28·5 °C, approximately 50 daughters from each cross were dissected to estimate its percent dysgenic ovaries.

mutation in the Nasr'Allah population seems to be stable over time.

4. Discussion

These results clearly show a stability over a five year period of the cytotype polymorphism previously observed by Anxolabéhère et al. (1982*b*) in Nasr'Allah population. Similarly and in a linked fashion, the ability to produce high rates of mutation at P element target loci is also present over this period.

This situation appears to be paradoxical given the properties of the P–M system: M cytotype always tends to disappear rapidly after the appearance of P elements in the *D. melanogaster* genome (Engels, 1979). Many studies of experimental populations indicate that the switch towards the P state generally requires between five and fifteen generations (Kidwell *et al.* 1981; Anxolabéhère *et al.* 1986). Data concerning the temporal and geographical distribution of samples collected in various locations show that a total switch of cytotype towards the P state in most of the populations of France studied took place in less than five years (Anxolabéhère *et al* 1982*a*, 1984).

Mukai *et al.* (1985), in a temporal study of lethal and detrimental load in Japanese populations, observed a dramatic increase in these two measures, at the same time as P elements penetrated populations in this part of the world. Further, the mutation rate began to fall back four years leater, approximately at

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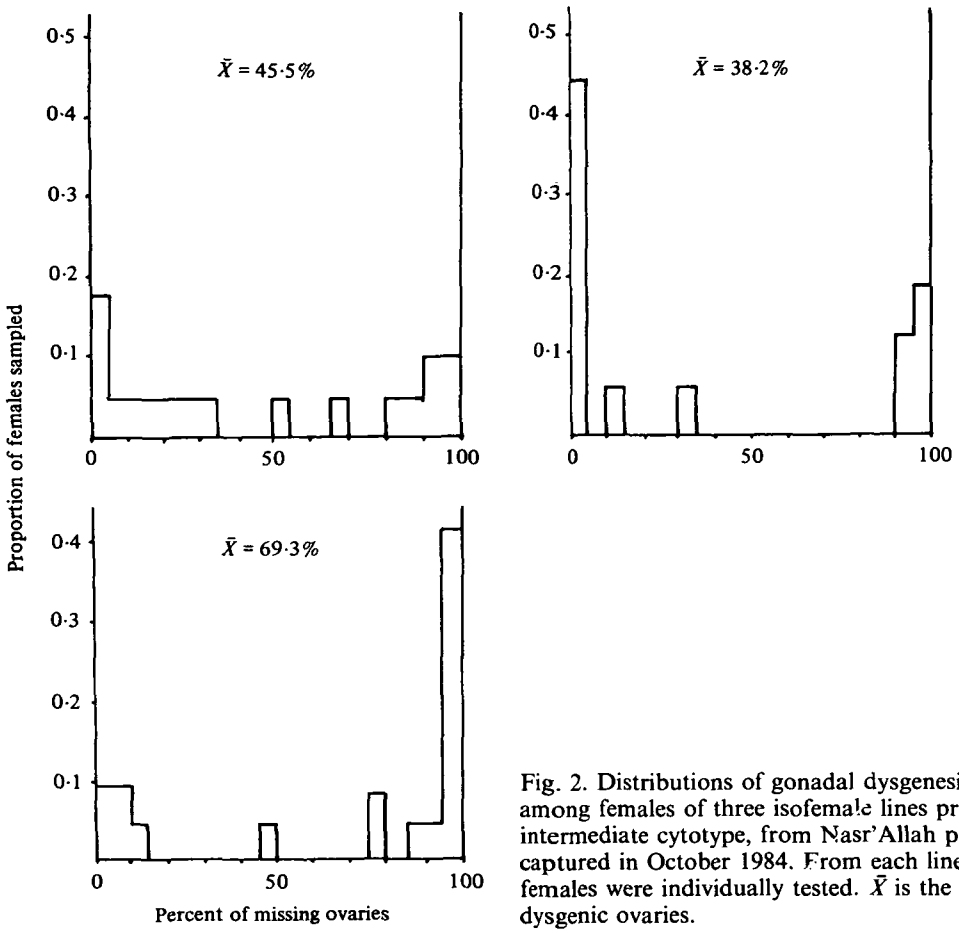

Fig. 2. Distributions of gonadal dysgenesis potential among females of three isofemale lines presenting an intermediate cytotype, from Nasr'Allah populations captured in October 1984. From each line, 20 virgin females were individually tested. $\bar{X}$ is the mean percent dysgenic ovaries.

the same time as the switch of the cytotype towards the P state in these populations. Mukai concluded that the high mutation rate period was transient, beginning with the invasion of the genome by the transposable elements and finishing with the cytotype switch. It is possible that the increase in mutation rate may only be a short-term phenomenon given that the cytotype switch is generally rapid.

The situation in Nasr'Allah is therefore particularly interesting, both with regard to the P–M system and with regard to genetic polymorphism. No such cytotype polymorphism stability has previously been reported. The mean cytotype level in this population seems to have remained more or less constant (between 70% and 80%) since 1979 (for this last year of capture, a mass-culture was tested in 1981: the cytotype level was 79%). No evolution of cytotype has been observed in this period of seven years. Moreover, the number of P copies detected by *in situ* hybridization on larval polytene chromosomes also appears to be stable (1979: $n = 35{\cdot}8 \pm 3{\cdot}6$, 1983: $n = 31{\cdot}3 \pm 3{\cdot}1$, 1985: $n = 34{\cdot}7 \pm 6{\cdot}3$, 1986: $n = 38{\cdot}1 \pm 7{\cdot}2$; mean of at least five larvae analysed for each year). There is no direct relationship among isofemale lines between the cytotype polymorphism and the polymorphism of the number of copies detected by *in situ* hybridization (Ronsseray & Anxolabéhère, 1986). The different years are not significantly different for the number of copies when compared by an analysis of variance. It seems that the Nasr'Allah population has reached an equilibrium with regard to the P–M system, with the simultaneous presence of both P and M cytotypes. The high rate of mutation induced by the P elements is therefore expected to continue over a long period in this population. This is the opposite of the situation observed by Mukai *et al.* (1985).

The presence of both P and M cytotypes in offspring of isofemale lines presenting an intermediate cytotype suggests the segregation of two kinds of element for determination of cytotype in this population. The bimodal distribution obtained from the Nasr'Allah population is probably due to the presence of the two kinds of element. The proportion of isofemale lines, presenting respectively P and M cytotypes, is correlated with the proportions of these P elements in the population as a whole. Kidwell (1985) studied a Spanish population, known to be an M′ type: she observed an unimodal distribution which suggests the existence of qualitative and/or quantitative differences in the P elements present in this population and in that of Nasr'Allah.

The temporal stability of cytotype polymorphism does not seem to be particular to the Nasr'Allah population. We are still studying strains from several European locations which show such cytotype polymorphisms (data not shown). As we report here, this phenomenon appears to be stable over a number of years.

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