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HYBRID DYSGENESIS IN TWO NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER.*

Ivana Tomišić[1], Marina Stamenković-Radak [1,2], Mirjana Miletić and M. Andjelković [1,2], *[1]Institute for Biological Research, „S. Stanković" [2]Faculty of Biology, University of Belgrade,* 11000 Belgrade, Yugoslavia.

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The moderately repetitive fraction of DNA includes a variety of sequences named transposable elements (TE) and represents the dynamic component of the genome in almost all species. Depending on which parts of the genome they transpose, TEs may induce mutations. Therefore, they contribute to the evolutionary process as an additional source of genetic variation in populations, and their long coexistence in the genome is accompanied by complex host-element coevolution (B e r g and H o w e 1989; W o o d r u f f 1992; P i n s k e r *et al.* 1993).

About one tenth of the *Drosophila* genome exists in the form of dispersed, moderately repetitive sequences, which include mobile elements that belong to different families (*for reference see* E n g e l s 1996). *Drosophila* populations are polymorphic for the P element family, heterogenous in size (0.5-2.9 kb). They replicate *via* a DNA intermediate, by nonreplicative transposition, leading to numerous phenotypic effects in interstrain hybrids. These occur when the P element is activated to transpose and replicate, under certain conditions, in germline cells of progeny, from a cross between females devoid of any P element (M strain) and males carrying P element (P strain). The phenomenon, called hybrid dysgenesis, includes high frequencies of gonadal sterility, mutations, recombinations in males, chromosome rearrangements, nondisjunctions and breaks (K i d w e l l 1986).

In the P-M system, two broad categories of individuals could be described as P and M strains. P type individuals carry an autonomous P element, whereas M type individuals have no P element. Additionally, in the M (pseudo-M) class, the subset of the M category, and the Q class, the subset of the P strains, the number of P element copies *per* haploid genome is low, 22-40 copies on average, most of which are defective and act as repressors (A n x o l a b é h è r e *et al.* 1985; B o n n i v a r d and H i g u e t 1999).

Surveys of natural populations of *D. melanogaster* in 1980s have revealed significant geographic differences in the presence of the P element. The strains derived from populations sampled in America, central Africa, north-eastern coast of Australia, eastern Asia and north-western Europe have been determined as P or Q (A n x o l a b é h è r e *et al.* 1984; 1985). In the rest of Eurasia, northern Africa and south-eastern coast of Australia, populations have been generally found to be M'. From western Europe and eastern Asia, where most strains are Q or P, an increased susceptibility is found towards mid-Asian and central Europe area, where M' strains predominate. The invasion pattern of P elements throughout *Drosophila* populations and the stability of the P element in the samples maintained in laboratories, are the subject of many studies and several hypotheses about the origin of the mobile elements and their evolutionary significance in populations (K i d w e l l 1994).

In the present paper, two populations of *D. melanogaster* from southern part of Central Europe were analyzed for the P-M status. The aim was to determine whether the status of a population, defined in 1982 as M', has changed, and to detect if the P-M status of the population maintained under laboratory conditions for several years differs from the sample recently collected from the field.

Two populations of *D. melanogaster* were studied for gonadal sterility using strains derived from large samples (several hundreds of flies) collected from two geographically close localities in Serbia: Slankamen (1995), 50 *km* north from Belgrade, and a Belgrade sample collected in 1999., from the garden in a residential area, 2 *km* from the city center.

Canton-S (C-S) and *Harwich* (Hw) laboratory strains of *D. melanogaster* were used as reference strains for determining the P-M status of the strains under study. Canton S (C-S) is a true M strain, whereas *Harwich* is a strong P strain (*obtained from M.G.Kidwell, University of Arizona*).

Characterizing individuals or populations in the P-M system was done by using specially designed crosses (K i d w e l l 1986). The cross of type "A", in which females of an M strain are crossed with males under test, measures the P factor activity, *i.e.* the ability to induce gonadal sterility. In the cross designated as "A*", the P susceptibility of the cytotype is measured (permissive state that allows induced gonadal sterility). Gonadal dysgenesis (GD), the absence of one or both ovaries in the progeny of such crosses, is used to indicate the hybrid dysgenesis.

For each population under study, two types of crosses, with P and M reference lines, were made in 3 replicas of mass mated 20 pairs *per* cross. The control cross was set between reference strains, *Canton-S* females and *Harwich* males. Prior to experiment the strains were kept as mass cultures under standard laboratory conditions, at 23^{0}C, on a cornmeal-sugar-yeast-agar medium. All experimental crosses were put at 29^{B}C.

The parents were removed after 3 days. A couple of days after the onset of emergence, F1 flies were transferred to a fresh medium and allowed to mature for two days at room temperature before dissection. Dissection of 50 F1 females from each cross allowed the assay of gonadal sterility, by determining the presence/absence of one or both ovaries.

To determine the P-M status of the tested populations the percentage of GD sterility in both crosses was considered (K i d w e l l 1986; B o n n i v a r d and H i g u e t 1999). As true M strain we considered one which gave none of the dysgenic ovaries in cross A and all dysgenic ovaries in cross A*. The ones that produce less than 10% GD in cross A* and not over 20% in cross A, we assumed to be P strains. Strains defined as Q did not produce GD in any of the crosses (or produced very low GD in both crosses). The so-called pseudo-M (M') strains gave less than 100% GD in cross A* and no GD in cross A. Variations within these classes are defined as "strong" and "weak.

The results are presented in Table 1. The gonadal sterility calculated from these data was 74% for the Slankamen population, and 87% for the Belgrade population. This shows that both popu-

lations are characterized as M type, with intermediate to strong P susceptibility. These data correspond to the results obtained for Krško (Slovenia), Divčibare (100 *km* SW from Belgrade), Slankamen as well in other populations in Central Europe surveyed in Anxolabéhère *et al.* (1984).

Table 1. P activity and P suceptibility of D. melanogaster strains estimated by the percentage of induced gonadal sterility (GD) in 50 dissected females from each cross

testcross	females	males	number of normal ovaries			%GD
			0	1	2	
cytotype test A*	Slankamen	Harwich	64	10	16	74
P test A	Canton-S	Slankamen	0	0	100	0
cytotype test A*	Belgrade	Harwich	78	9	4	87
P test A	Canton-S	Belgrade	0	0	100	0
control test A	Canton-S	Harwich	100	0	0	100

Our results show that the M' status of the Slankamen population did not change after more than 15 years. Results of Bonnivard and Higuet (1999) on phenotypic properties using the GD test and molecular characterization *via* the estimation of element sizes, show that the geographical distribution of P, Q and M' populations has been highly stable, and that the European longitudinal gradient observed in early 1980s still exists. This stability is in contrast with the fast invasion of the P element throughout the world since 1950s, and could be due to the presence of Q populations in Europe, which act like a buffer zone between P and M' populations, slowing down the P element invasion. Under this "buffer" model, migration of individuals from P and M' populations is limited to Q populations which can repress transposition and maintain the Q phenotype. Only migrants coming from Q populations are received in P and M populations without disturbing their P or M' status. Such buffer zones are recognized also in China, north-Africa and eastern Australia. No such zone is found in America, where only P and Q populations are detected in the 1980s. In those areas, the hypothesis proposed by Kidwell (1983), suggests that random processes of internal deletions in P strains are leading to Q, and later, to M' status, and the heterogenous geographical distribution results from a balance between migration and drift.

Some authors suggest that the loss of P factors is stochastic and irreversible (Engels 1996), and, as such, expected in laboratory populations, while others prefer the hypothesis that P factors occured in natural populations after laboratory stocks were started (Kidwell 1994). Laboratory stocks of Japanese origin of different age (maintained for 8-27 years in laboratory conditions) were examined to see if they retain the P-M status (Yamamoto *et al.* 1984). As in the majority of similar surveys, the results were ambigous. Both populations analyzed in our study are of the same P-M category, although the Belgrade sample spent only two generations under laboratory conditions before the experiment, and Slankamen population was maintained in laboratory for almost five years before the analysis. This suggests that P-M polymorphism may remain stable under laboratory conditions. However, further study is needed to determine whether the higher percentage of gonadal sterility in the Belgrade population would persist on the temporal scale. Alternatively, it could be expected to decrease to some degree, in time, before reaching a stable frequency.

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