Ivanov, Yu.N., and A.V. Ivannikov. Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk, 630090, Russia. FAX: (3832) 35 65 58. Difference in mutation rates between flies of small and large body sizes in natural *Drosophila melanogaster* populations and regulatory meaning of this phenomenon.

Flies often differ in body size within *D. melanogaster* populations, larger individuals weighing up to 5 times as much as their smaller mates. We have found that these additionally differ in the frequency, at which lethal chromosomes 2 and MR-factors occur in them. Our intention was to answer the question as to whether there is a difference between the size different flies in the frequency at which mutations occur in their gametes.

Measuring the rates of spontaneous mutation in X chromosome of males from four Altai populations, we visually classed the males into small, medium and large. The results of a comparative study on the mutation rates in X chromosome with respect to the size groups are presented in the Table. Of the seven mutations arisen in 4770 gametes, six were confined to the small, one to the medium, and none to the large males. Fisher's precise method, which we have herein applied for evaluating 2×2 tabular data instead of χ^2, reveals significant differences in mutability between the small and large males. Given the zero hypothesis (*i.e.*, that the mutation rates are all equal), the probability of the differences is somewhat low ($P = 0.0051$). On the face of it, the zero hypothesis was rejected for an alternative assumption that mutability is stronger in the gametes of small males than in those of large ones. Relating the medium-size males to the small or large ones, the differences still hold significant ($P = 0.0141$ and 0.0057, respectively).

Mutability should necessarily be elevated in small flies compared with large ones, because the mutation process acts as a factor that regulates the population density of the species. Larvae end up as small flies when the population density is high and as large flies when it is low. Therefore, the dimensions of a fly body are closely associated with population density. If it is high (*i.e.*, the flies are small), there should be factors acting in a manner to restrict the population growth. Really, females lay less eggs because they are small; mortality rates, accounted for by no matter which biocoenotic factors, increase; additionally, we have found that the mortality rates increase due to elevated mutability rates. Decomposing the spontaneous mutation process in the *D. melanogaster* genome into the basic types of mutations (Ivanov, 1991) yields a zygote mortality rate accounted for by dominant lethals in the population as follows: $S = 13.5u - 45.5u^2$, where u is the mutation rates in X chromosome. As can be seen, S is nearly in a direct proportion to mutability u. This value of S is by far not low, as even at normal mutation rates ($u = 0.3\%$) dominant lethals make up as much as 4% of all zygotes. In contrast, at a low population density (*i.e.*, when flies are large) the effect of all these population size-restricting factors is attenuated, which urges the population to grow in abundance.

In connection with the regulatory role of the mutation process, the significance of redundant DNA in the genome and the causes of non-adaptive karyotype structure in many species become clear. The DNA redundancy exists in the form of 1) blockwise and 2) intercalar heterochromatin (between genes), as well as in the form of 3) introns (inside genes). Heterochromatin is represented by genetically inactive (empty) DNA sites from repeated nucleotide sequences or "repeats".

Dominant lethals (DLM) are divided into two types: 1) chromosomal and 2) genic. In *D. melanogaster*, chromosomal DLM make up 95%, and genic ones 5% of all the DLM (Ivanov, 1991). Chromosomal DLM are simple disruptions of chromosome arms with a loss of acentric fragments in the course of subsequent cell division, *i.e.*, terminal deletions. Genic DLM are ordinary recessive lethals with some penetrance in heterozygotes.

Chromosomal DLM are a factor determining the number of chromosome arms in the species karyotype. This followed from the fact that the mean number A of chromosomal DLM in the genome is a decreasing function of the number f of chromosome arms: $A = k(1 - \alpha f)$ where k and α are positive constants. From the formula one can see that as the number f increases, *i.e.*, as karyotype is fragmented into progressively smaller chromosomes, the number A of chromosomal DLM decreases and becomes zero as soon as the equality $\alpha f = 1$ is reached. On the contrary, in order that the number of DLM in the genome increases, it is necessary to diminish the number f, *i.e.*, the genome becomes more vulnerable to chromosomal DLM at a small number of arms. This is connected with the fact that the genic contents of the arms increases and their losses become more dangerous.

The presence of heterochromatin (repeats) in chromosomes makes the genome vulnerable to DLM, since it increases the length of the chromosomal DNA thread as the size of the target hit by mutations. In cytogenetics, it is well known that structural damages are distributed along the chromosome nonuniformly. It is noteworthy it is just heterochromatin regions that are characterized by an increased vulnerability (Bochkov *et al.*, 1984). Thanks to this, the species has the capacity of regulating its numbers by means of changing the mutation rate. Indeed, in order that the mutability can be a regulator of the population size, an effective increase of lethality is necessary through elevation of the mutation rate, which is achieved by means of increase of the genome vulnerability to DLM at the expense of repeats.

The intronic gene structure in eukaryotes has, *inter alia*, the meaning that introns, occupying up to 50% and more of the gene length, elongate thereby, similarly to the intercalar heterochromatin, the DNA thread and heighten the DLM probabiltiy, which is necessary for limitation of the species abundance.

The structure of the genome in *D. melanogaster* in which the numbers are regulated by mutations is in a good accordance with these notions and thereby confirm them.

1) The *D. melanogaster* karyotype consists only of 4 chromosomes, the number of arms being no more than 8. The genetic contents of the arms is very large and their breaks are very effective as DLM whose proportion is about 54% of all spontaneous mutations in the genome (Ivanov, 1991). As a contrast, the grayling (*Thymallus thymallus*) karyotype may be considered where there are 100 - 106 chromosomes and 170 chromosome arms (Severin, 1979). DLM in the grayling do not play practically any role, because the genetic contents of each of so numerous arms is negligibly small, and their breaks and losses are not dangerous and do not represent DLM, which is confirmed by the variation of the chromosome number in the karyotype.

2) Blockwise heterochromatin occupies about a third part of the whole *D. melanogaster* genome. In man (*Homo sapiens*) the fraction of DNA with repeats makes up 36% of the whole DNA, and is therein two times larger than the blockwise heterochromatin (Bochkov *et al.*, 1984). It is possible that in *D. melanogaster* this fraction is also considerably larger than that of blockwise heterochromatin and sometimes exceeds 50%, which is what determines the high DLM level.

3) Hetero- and euchromatin are distributed in chromosomes in a way that the probability of the genome being hit by DLM is maximal. The euchromatin is located in distal, and the heterochromatin in proximal, regions of chromosome arms. That is why in any large chromosome the region whose breaks are lethal occupies practically the whole chromosome, which would not have been the case, if the heterochromatin had been localized distally.

Table 1. Frequency of occurrence of X-linked recessive lethal and visible mutations in the gametes of males with different body size from four Altai populations of *D. melanogaster*, September 1992.

Male group by size	Average male weight of the group over all populations, mg	n	N	*u*, %
Small	0.26 - 0.53	6	1541	0.389
Medium	0.78 - 0.79	1	1054	0.095
Large	0.74 - 0.90	0	2175	0

n is the number of mutations; N is the gamete sample size; *u* is the frequency of occurrence of mutations

In this way, the total length of the chromosomal DNA thread in the *D. melanogaster* genome exceeds by more than two times its unique (translatable) part, the size of arms and localization of blockwise heterochromatin in them being such that the chromosomal DLM probability is maximal.

The modus operandi of mutability is consistent with the basic principle of life organization, of which the crux is not the maximization of an individual's fitness, but allocentrism, *i.e.*, pursuing collective interest of all the species within the ecosystem. Mutability is liable to change adequately to the population density in order to even out fluctuations in the species population size. The agents that promote mutability (as long as this is a necessary requirement for lowering the population size for the sake of ecosystem stability) may be any factors of genetic instability (plasmids, viruses or other mobile genetic elements) which are activated by metabolites, typical of the larvae and flies on the overpopulated substratum. The body of evidence is now great that such agents are primarily MR-factors that are quite frequent in all populations (Hiraizumi, 1971; Thompson and Woodruff, 1978; Green and Shepherd, 1979).

In the case of the tolerant (non-mutant) forms, the selection magnitude changes depending on their frequency, so that selection maintains a stable polymorphism in the population (Luchnikova, 1978), and in the case of mutant forms, the selection rapidly eliminates them, *i.e.*, generally speaking, is a kind of guardian of species permanence (Jenkin, 1867; Agassiz, 1874; Danilevskiy, 1885). The intraspecific competition is an allocentric property of species, since it is exacerbated in overpopulation and limits thereby the species abundance, protecting the ecosystem from degradation. It is now becoming clear that the mutation process is not a factor of transmutation (speciation) and biogenesis, either, but, similarly to selection and competition, exerts regulatory functions in the ecosystem.

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Kim, W., J.M. Kim, and D.J. Shin. Department of Biology, Dankook University, Cheonan-Si, Choong-Nam 330-714, Korea. Molecular analysis for specific *hobo* deletion derivatives in the Korean population of *Drosophila melanogaster*.

On the basis of our results of *in situ* and Southern blot hybridization using *Xho*I restriction enzyme (Kim and Kim, 1996), the Korean population of *D. melanogaster* appeared to have a low copy number of 3.0 kb putative full-size *hobo* elements and a high copy number of internally deleted *hobo* elements. This result is somehow comparable with the earlier reports detected from American and Eurasian populations of *D. melanogaster* (Periquet *et al.*, 1989a, b; Pascual and Periquet, 1991; Boussy and Daniels, 1991). Periquet *et al.* (1989a, 1990) reported the presence of two major classes of *hobo* elements, a 3.0 kb element class and one particular deletion derivative class of elements called the *Th* element, which have accumulated in all naturally-occurring strains throughout the Eurasian continent. They suggested that the presence of *Th* element might be interpreted as potential regulatory elements of the *hobo*-induced hybrid dysgenesis.

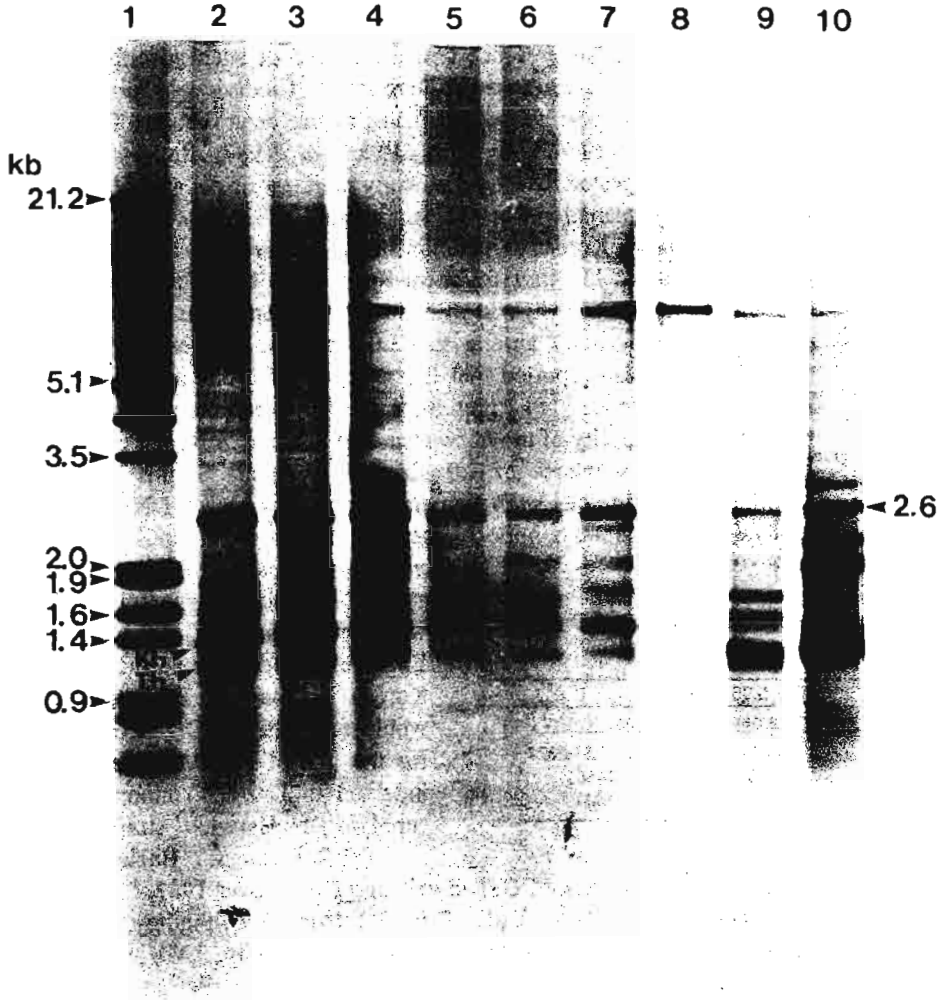

Figure 1. Southern blot analysis for the structure of *hobo* elements from Korean lines of *D. melanogaster*. Genomic DNAs were digested with *Xho*I, and hybridized with the 2.6 kb *Xho*I restriction fragment of the pH108 plasmid as a probe. Lanes are as follows: (1) Dig-labelled DNA marker III, (2) Cheonan 96-33 (H^+), (3) Cheju 96-29 (H^+), (4) Cheonan 96-15 (H^0), (5) Cheju 96-12 (H^0), (6) Cheonan 96-6 (H^-), (7) Cheju 96-9 (H^-), (8) Basc (E), (9) Harwich^Y (E), (10) 23.5*/Cy (H). H^+, H^0, and H^- strains were classified by reference tests of Pascual and Periquet (1991).

Based on the result of Southern blot hybridization, a specific 1.7 kb *hobo* deletion derivative (1.3 kb *Xho*I restriction fragment in Figure 1) is the most preserved in all of the Korean lines tested and is termed *Kh* element. The 1.5 kb *Th* element, giving a 1.1 kb fragment and 3.0 kb full-size *hobo* element (2.6 kb fragment) are also observed in these lines (Figure 1). The entire 1.7 kb sequence of four *Kh* elements derived from Korean lines have been obtained by polymerase chain reaction (PCR) and DNA sequencing. PCR amplification of *Kh* element sequence was performed on the genomic DNA using the following two primer sequences in pH108 (Streck *et al.*, 1986): #1, 5'-CAGAGAACTGCAAGGGT GGC-3' (1-21), and #2947,