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Mutation Processes in Natural Populations of *Drosophila melanogaster* and *Hirundo rustica* from Radiation-Contaminated Regions of Ukraine

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Abstract—Natural populations of *Drosophila melanogaster* and *Hirundo rustica* from regions of Ukraine exhibiting different levels of radiation contamination are investigated. Genetic monitoring was performed with respect to such parameters as the frequency of visible sex-linked mutations, frequency of gonad reduction in *Drosophila*, and rate of interphase manifestations of chromosomal instability in the erythrocytes of the birds. The results attest to a possible opposite dependence of the level of chromosomal instability among swallows and that of the rate of lethal mutations in the sex chromosome of *Drosophila* on the density of radiation contamination.

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INTRODUCTION

More than 20 years have passed since the accident at the Chernobyl nuclear power plant. An enormous quantity of scientific studies have been carried out in the course of these years by scientists from different countries along the most varied directions for the purpose of answering a question of the greatest importance for mankind: What are the consequences of this extraordinary event? The official position of the United Nations [1] is that the consequences of the accident for human health have turned out to be significantly less than expected. An increase in the animal population has been observed in the exclusion zone of the Chernobyl nuclear power plant and this was interpreted as a reduction of the natural ecosystems. Such opinions arose for several reasons: (1) Most studies have been performed under laboratory conditions, which means that their results cannot be simply translated to populations processes, hence for precisely this reason they were not included in the reports of governmental organizations [2]. (2) Classical model subjects were not made sufficient use of in radiobiological investigations, whereas it is only with the use of exhaustively studied species of living organisms that it is possible to obtain reliable results (mice constitute an exception here [3]). (3) The results of scientific studies are often contradictory, which may be explained by the features of the study subjects and the diversity of the techniques employed [4].

Vast regions of the territory of Russia, Ukraine, and Belarus as well as other European countries were affected by contamination produced by long-lived radioactive isotopes as a consequence of the accident at the Chernobyl nuclear power plant. This means that most of the animal and plant populations as well as the human population are living under conditions of chronic low-dose radioactive contamination, the genetic consequences of which have not been studied at all, while the results for those species which have been studied have been contradictory.

The objective of the present study was to investigate mutational processes in natural populations of model organisms (*Drosophila melanogaster* and *Hirundo rustica*) that reflect the genetic consequences of chronic low-dose irradiation in conjunction with other factors of environmental pollution.

MATERIALS AND METHODS

Drosophila melanogaster is a "cosmopolitan" species commonly present at sites where subject matter is collected as well as a classical model subject. Under laboratory conditions the fly is bred in a standard medium [5]. At optimal temperatures, the length of development of an individual, from the egg phase to the imago, is roughly two weeks.

The forest swallow, *Hirundo rustica*, a small bird (≈20 g) from the order *Passeriformes*, feeds on insects, principally *Diptera* and *Hymenoptera*, which are captured in the summer. The males and females construct nests on (or within) man-made structures and the females usually lay 4 or 5 eggs. More than half the females may lay a second clutch of eggs. Forest swallows live in isolation or in colonies that may exceed 120 pairs. The forest swallows of Ukraine winter in southern Africa, where complete molting takes place.

Blood masks of the swallows and Drosophila individuals were obtained in the summer of 2005. Regions with different levels of radiation contamination were selected for the study. Thus, flies were collected at three sites near the Chernobyl nuclear power plant with level of radioactive contamination 5, 12.5, and 55.5 Cu/m^2, respectively, as well as in the cities of Kiev, Odessa, Uman', Lubny, Piryatin, and Bologna (Italy).

The swallows were trapped within the territory of three villages in Kiev province that differed in terms of radioactive contamination: (1) Pesky Village, Ivankov District, at the border of the Chernobyl exclusion zone (48 birds), with mean density of contamination with radioactive isotopes with respect to the three radioactive isotopes ^{137}Cs, ^{90}Sr, and ^{239}Pu (151, 47.7, and 0.909 kBq/m^2, respectively); (2) Dityatki Village, Ivankov District, also at the border of the Chernobyl exclusion zone (44 individuals), with mean density of contamination with respect to the isotopes ^{137}Cs, ^{90}Sr, and ^{239}Pu (148, 40.3, and 0.557 kBq/m^2, respectively); and (3) Zhovtneve Village, Borodyan District, selected as a control, or provisionally "clean" region (21 birds), with mean density of contamination with respect to the isotopes ^{137}Cs, ^{90}Sr, and ^{239}Pu (26, 4.7, and 0.084 kBq/m^2, respectively). A total of 113 birds were selected for the study.

Visible mutations were studied by examining five generations of inbred lines obtained by means of individual crossings of 30 females from each natural population. In order to estimate the spontaneous level of lethal sex-linked mutations, males from the natural populations were crossed with females from the line *C(1)DX* and the sex ratio in the first generation compared to the control ratio according to the chi-square method [6].

The presence of hybrid dysgenesis was determined by means of gonadal dysgenesis assay (GD) on the basis of the state of extracted gonads by visual evaluation of the degree of development of the gonads. Only unilateral and bilateral. reduction of the ovaries and testes were included in the assay. A total of 50 individuals of each sex for all the populations and the laboratory line *Canton S.* were analyzed. The GD percentage was calculated according to the formula

$$\%GD = S\ \%GD(1) + \%GD(2),$$

where %GD is the percentage of individuals with a single reduced ovary (respectively, testis) and %GD(2), the percentage of individuals with bilateral gonad reduction.

Males aged 24 h were selected to estimate the resistance of the flies to the effect of radiation over the course of five generations of laboratory culturing of flies from the populations at the Chernobyl plant (55.5 Cu/m^2) and from Bologna. These male individuals were subjected to gamma irradiation at a dose of 30 Gr, which it is believed induces the maximum quantity of changes in the genome with minimal mortality of the organisms. The radiation power amounted to 0.017 Gr/s at room temperature (Issledovatel' gamma radiation device, ^{60}Co source). The irradiated males were then crossed with females from the line *C(1)DX* and the sex ratio estimated in the first generation; the ratio was compared with the control according to the chi-square method.

Blood was taken from the axillary vein. The masks were dried in open air and stained for 5 min in a prepared May-Grunwald stain fixative. Final staining was performed in a 4% aqueous solution of Romanovskii-Giemsa stain for 40 min. With such a combination it became possible to obtain good staining of the nuclei and differentiated staining of the granules in the granulocyte.

The frequency of erythrocytes with micronuclei (MR) and the following two nuclear anomalies which we have described previously [7] were identified under the microscope: "caudate" nuclei (CN) and "sprouting" nuclei (SN). Bilaciniate nuclei (BN) and binuclear erythrocytes (BE) were selected as indicators of disturbances in mitosis and were combined into a single parameter in view of the strict interdependence of changes in these markers. Micronuclei, caudate nuclei, sprouting nuclei, and binuclear cells are classical indicators of chromosomal instability and are therefore recommended for estimating this parameter [8]. A total of 10000 erythrocytes in each sample, which included only mature and undamaged erythrocytes, were counted.

Statistical analysis was performed by means of standard methods [6].

RESULTS AND DISCUSSION

Samples were collected in different regions in Ukraine, the size of each of the samples corresponding to the population density of *D. melanogaster* at these sites (cf. table). The sample of the Italian population was represented by flies that had been sent for the analysis and it is possible that they do not reflect the actual parameters of this population.

In neither of the natural populations were visible mutations discovered. On the one hand, this may be related to the size of the samples, since according to the data of Gershenzon [9], the frequency of visible mutations in the Kiev population in the 1937–1947 period amounted to 0.25% and, consequently, our sample may have been too small. On the other hand, the frequency of visible mutations in the population of the city of

Uman' varied in different years, from 0.17% (1930–1940) [9] to 0.9% (1981–1991) [10] and, consequently, this leads us to conclude that in 2005 the frequency of visible mutations in this population was less than in the previous years. It may be suggested that at the time of the study these populations were not in a state of mutational outbreak.

It is known that 80% of all spontaneous mutations in *D. melanogaster* are caused by the activity of mobile elements [11]. The rate of reduction of gonads in Drosophila is one indicator of this activity. In our studies this indicator varied from 0 (which meant that we could not consider the indicator if it was less than 1%) to $23 \pm 6\%$. Such a level of reduction of the gonads lead us to conclude that in all the populations studied, including the laboratory line of the wild type *Canton S.*, we had not observed any activity of the mobile elements, though it should be noted that the phenomenon of gonad reduction is itself more often found in females in the Odessa, Chernobyl, Piryatin, and Lubny populations.

The result of an analysis of the reduction in the gonads of both sexes provided indirect confirmation of the low frequency of visible mutations in the populations being studied, since it had been previously demonstrated for the Uman' population that mutation outbreaks are accompanied by the involvement of mobile elements (*P, hobo*) at particular loci of the genome of *D. melanogaster.*

The next stage of the study was to investigate the level of lethal mutations in the X-chromosome of Drosophila. Our results show that the level of sex-linked lethal mutations exceeds statistically reliably the control ($\chi^2_{(\text{Lubny})} = 5.4$, $p \geqslant 0.50$; $\chi^2_{(\text{Piryatin})} = 4.02$, $p > 0.50$) only in the natural populations of Lubny and Piryatin. In the populations of Kiev, Chernobyl, Uman', Odessa, and Bologna, the indicator was at the level of the control.

Males who were descendants of individuals that had been collected in Chernobyl and Bologna were subsequently subjected to acute irradiation with gamma rays at a dose of 50 Gr. They were then tested for the presence of lethal mutations in the X-chromosome. It turned out that a statistically reliable increase in the level of lethal mutations was observed in individuals from the population of Bologna, whereas no such increase was observed among individuals from the Chernobyl population.

In the transition to culturing of populations, an increase in the frequency of mutations in the generations was observed, moreover, a monotonic increase in this indicator was observed in two of the populations (Piryatin, Chernobyl, 55 Ci/m^2). In the other populations this process was intermittent. Thus, besides generations in which an increase in the frequency of mutations was observed, there were also generations in which no mutations at all were detected. It should be emphasized that in the fifth generation the frequency of mutations in the Chernobyl population (55.5 Ci/m^2) had grown fully ten-fold (Fig. 1).

Number of *Drosophila melanogaster* individuals captured in Nature

Natural Populations	Males	Females
Kiev	208	138
Uman'	478	704
Odessa	621	497
Chernobyl (city), 5 Ci/m^2	80	70
Chernobyl, 12.5 Ci/m^2	34	51
Chernobyl, 55.5 Ci/m^2	41	35
Lubny	226	597
Piryatin	484	723
Italy	139	94

The following mutations were found among Drosophila individuals in all the populations studied: changes in eye color; disturbances in sclerotization of the abdomen; blisters on the wings; and changes in venation. It should be noted that the range of mutations in the Kiev population was basically identical to that described by Gershenzon et al. [9], though we did not observe certain types of mutations in the course of five generations of inbred crossing. Thus, we found a contraction of the range of eye color and a decrease in the diversity of changes in the venation of the wings. The mutations *yellow, singed*, and *abnormal abdomen* have been previously described in several studies [10, 13–15] for the Uman' population, though in our experiments we did not discover individuals with similar changes in pheno-

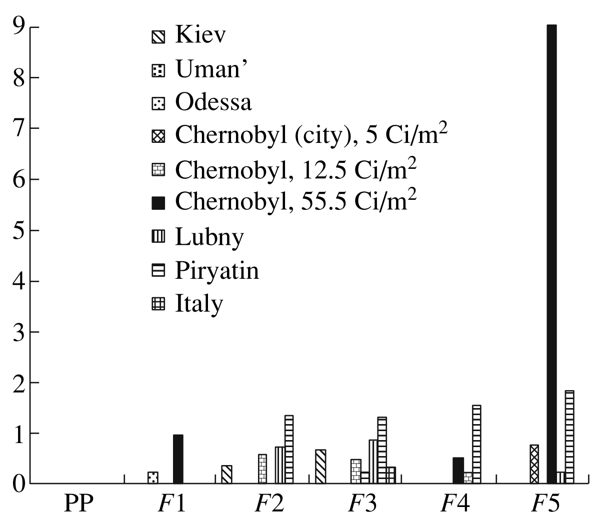

Fig. 1. Frequency of mutations (along the vertical, %) in five generations of laboratory culturing: PP, parent generation; *F*1–*F*5, daughter generations.

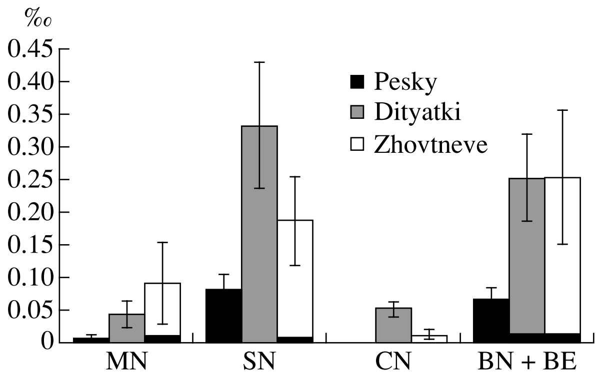

Fig. 2. Levels of parameters of chromosomal instability among swallows in areas with different levels of radiation contamination: MN—micronuclei; SN—sprouting nuclei; BN—bilaciniate nuclei; CN—caudate nuclei; and BE—binuclear erythrocytes.

type in any of the generations in the course of culturing of the Uman' population.

The frequency of all the parameters of chromosomal instability studied here in the swallows were found at a rather low level, which is characteristic of all the species of birds we have previously analyzed [16]. In most of the studies micronuclei represented the basic interphase indicator of the presence of a mutagenic load and, correspondingly, level of chromosomal instability. The value of this indicator amounted to 0.007 ± 0.004% in the population of swallows from an intensively contaminated region (Pesky Village), and 0.043 ± 0.020% from a moderately contaminated region (Dityatki Village), whereas in the population of birds from a provisionally clean region (Zhovtneve Village), it reached 0.090 ± 0.062%. Despite the clear decreasing trend in the frequency of micronuclei with an increase in the degree of contamination, the differences between the populations with respect to this parameter were not statistically significant (Fig. 2).

Additional parameters that reflect the presence of a chemical (sprouting nuclei) or physical (caudate nuclei) mutagenic load were also estimated in three populations of swallows. Swallows from Pesky Village were characterized by the lowest level of these parameters (Fig. 2). The differences between these parameters were statistically reliable for both parameters ($p < 0.05$) when compared with values obtained for birds from moderately contaminated regions (Dityatki Village) and were comparable with values obtained in an analysis of a population of birds from the provisionally clean region (Zhovtneve Village). In comparing the swallows from the villages of Dityatki and Zhovtneve, a statistically significant increase in the level was observed only with respect to CN (0.052 ± 0.010%, Dityatki; 0.010 ± 0.010%, Zhovtneve, $p < 0.05$). There were no differences with respect to SN. A analogous law was noted in comparing the indicators for disturbances in mitosis (BN + BE). Thus, reliably reduced rates of this parameter were characteristic of the population of swallows from Pesky Village; swallows from the villages of Zhovtneve and Dityatki exhibited similar frequencies of manifestation of disturbances in mitosis (Fig. 2). We adopted the frequencies of bilaciniate nuclei and of binuclear erythrocytes as indicators of disturbances in mitosis, since BN reflects amitotic divisions and BE, a disturbance in cytokinesis.

From the results that have been presented here it is clear that the erythrocytes of swallows from maximally contaminated regions are characterized by reduced (by comparison with the moderately contaminated zone) frequencies of interphase manifestations of chromosomal instability and possess levels of these indicators that are comparable to those of birds from the provisionally clean zone (except for BN + BE, $p < 0.05$).

We should note that in the moderately contaminated zone (Dityatki) the highest level of the marker of radiation effects, that is, the number of caudate nuclei by comparison with that in the highly contaminated zone, was observed. It may be suggested that this phenomenon is the result of practically complete elimination of radiosensitive individuals in the maximally contaminated regions.

Thus, our results attest to the possible existence of an opposite dependence of the level of chromosomal instability in swallows and that of the rate of lethal mutations in the sex chromosome of Drosophila on the density of radioactive contamination. The phenomenon has several possible explanations:

1. Rigid selection in the contaminated areas against individuals exhibiting an increased level of chromosomal instability and an increased level of lethal mutations.

2. Adaptation of organisms living in the contaminated areas to chronic doses of radioactive effects.

3. Migration of individuals with increased levels of chromosomal instability to cleaner regions (only for birds).

There are preliminary data that support the second and third explanations (Mousseau, Beckham, et al., unpublished data). But it must also be noted that the population of Drosophila from the region possessing the highest radiation contamination is characterized by the greatest mutation load. It may be suggested that the increased heterzygosity of this population also attests to increased adaptivity of the population.

We believe that additional studies of other regions, an analysis of several generations of birds and Drosophila, as well as studies of particular birds over the course of several years are needed to arrive at an unambiguous answer to these questions.

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