Genome Reduction in a Hemiclonal Frog *Rana esculenta* From Radioactively Contaminated Areas

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**ABSTRACT**

A decrease in genome size was found in the hemiclonal hybridogenetic frog *Rana esculenta* (R. ridibunda × R. lessonae) from areas of radioactive contamination that resulted from the Chernobyl fallout. This genome reduction was of up to 4% and correlated with the background level of gamma-radiation (linear regression corresponded on average to −0.4% per doubling of radiation level). No change in genome size was observed in the coexisting parental species *R. lessonae*. There was no correlation between genome size and body mass in *R. esculenta* froglets, which have metamorphosed in the year of the study. The hemiclonal forms may become a suitable object for study on biological significance of individual DNA sequences (and of genome size as a whole) because mutant animals with deletions in a specified genome can arise after a low radiation dose. The proneness to genetic damage makes such forms also a prospective bioindicator of radioactive (and possibly other mutagenic) pollution with the effects of genetic damage conveniently and rapidly monitored by DNA flow cytometry.

The hemiclonal (hybridogenetic, non-Mendelian) frog *Rana esculenta* L. arose from the original hybridization of *R. ridibunda* Pall. × *R. lessonae* Cam. (Berger 1968). In gametogenesis of this hybrid, one of the parental genomes is selectively eliminated before premeiotic DNA synthesis, and the remaining genome undergoes a compensatory reduplication followed by a “normal” meiosis, which results in gametes containing only one of the parental genomes (Uzzell et al. 1980; Tunner and Heppich 1981; Vinogradov et al. 1990, 1991). The hybrid is recreated in each generation by backcrossing to that parental species whose genome was eliminated. The DNA content of the *R. ridibunda* genome is greater than that of *R. lessonae* by 16%, which allows us to identify a set of genomes in somatic and germinal cells in any specimen of this hybridogenetic complex by means of DNA flow cytometry (Vinogradov et al. 1990, 1991). The direction of elimination shows a gene-dosage effect (in triploids that genome is eliminated that is in the minority) and is supposed to be determined by the ratio of activities of elimination-related factors in both genomes, which on average (over all the populations studied) is in favor of the *ridibunda* genome (Vinogradov et al. 1990, 1991). Both genomes are expressed in somatic cells of a hybrid (Uzzell et al. 1980). Other hybridogenetic complexes are known: e.g., fishes of the genus *Poeciliopsis* (Leslie and Vrijenhoek 1978, 1980; Quattro et al. 1991) and insects of the genus *Bacillus* (Giorgi 1992).

The hemiclonal genomes of *R. esculenta* and *Poeciliopsis* fishes are known to accumulate deleterious recessive mutations (Leslie and Vrijenhoek 1978, 1980; Graf and Pollis-Pelaz 1989). Such accumulation is expected in clonal genomes that always occur in heterozygous conditions without recombination (“sheltered” genomes). This was originally proposed for the evolution of the Y chromosome (Muller 1914; Nei 1970) and confirmed experimentally (Rice 1994). Furthermore, large decreases in DNA content (up to 50%) were found in the sperm of *R. esculenta* males from radioactively contaminated areas (Vinogradov et al. 1990), which suggests that genome reduction could appear in zygotes of this hybridogenetic form.

In the present investigation, frogs of the *R. esculenta* complex from the areas of radioactive contamination that resulted from the Chernobyl fallout were studied by means of DNA flow cytometry.

**MATERIALS AND METHODS**

Frogs were collected in August 1994 in the Bryansk region (Byelorussia) from three places with different background levels of radioactive contamination: environs of Sennoe village (γ radiation was 220 μR/hr), environs of Petryatinka village (γ radiation was 60 μR/hr), and, near the national nature reserve Bryansk Les, a control point without contamination (γ radiation was 15 μR/hr). The map is shown in Figure 1. Genome size was determined by means of DNA flow cytometry in blood cells as described previously (Vinogradov et al. 1990, 1991) and presented in relation to the genome of *R. temporaria* (RT-index), which was used as an internal standard. Triplicate
repeats of staining and measurements of cell samples from the individual animals gave SEs < 0.1%.

RESULTS AND DISCUSSION

We found a statistically significant decrease in genome size (of up to 4%) in R. esculenta from the radioactively contaminated points (Figure 2). All the populations were of the most common, diploid L-E type (i.e., R. esculenta coexists with R. lessonae, and the lessonae genome is eliminated). The genome size of the coexisting parental R. lessonae was not changed (Figure 2). The mean genome size of R. esculenta was log-linearly correlated with the background level of γ-radiation (Figure 3). The linear regression corresponded on average to −0.4% per doubling of radiation level.

The most plausible explanation for genome reduction in R. esculenta (and the absence of such reduction in the coexisting parents) is that these frogs resulted from fertilization with gametes having deletions in the clonal ridibunda genome (of up to 8%). The R. esculenta males, which produced sperm with ridibunda genome (in accordance with their population type) but had part of sperms with the decreased DNA content, were found in the L-E population from a radioactively contaminated area (Vinogradov et al. 1990). The decrease was much greater then (up to 50%), and it is unlikely that zygotes resulted from fertilization with those sperms would be viable. The γ-radiation level was also much higher in the previous study, conducted exactly 1 yr after the fallout (1.1 mR/hr; environs of Babichi village, Byelorussia, 130 km W-SW from the area of the present study). No change in genome size was then found in gametes and somatic cells of coexisting R. lessonae, nor in somatic cells of R. esculenta (born before the fallout), which suggests that the preferential damage occurred at gametogenesis in the clonal ridibunda genome. Such damage should then accumulate according to the expectation for the sheltered genome condition (Müller 1914, 1964; Nei 1970; Leslie and Vrijenhoek 1978, 1980; Rice 1994).

Owing to the increased vulnerability of a genome, its accumulation of genetic damage under a sheltered condition, and the outcome of its change in genome size, hemiclonal forms may become a prospective bioindicator of radioactive (and possibly other mutagenic) pollution with the effects of genetic damage convenient for rapid registration with DNA flow cytometry.

There was no correlation between genome size and body mass in R. esculenta froglets, which had metamorphosed in the year of the study, although body masses of animals from the point with the highest radiation level (Sennoe) seemed to be more scattered (Figure 4). This suggests that deletions of up to 4% of total DNA
where the association of deletions in satellite DNA with Communicating editor: Wu
Wu, C. I., J. R. True can arise after a low-dose mutagenic action, probably
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ual genomic elements (and of genome size as a whole) males: cytological evidence from DNA ¯ow cytometry. Genome
object for research on biological signi®cance of individ-
Vinogradov, A. E.,
Morell
1995, 1998). Note that, because selection pres-
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ecophysiological) concept of redundant DNA (Vino-
1994), up to the XO condition in some
hypothetical forms could become a suitable object for research on biological signi®cance of individual
genic elements (and of genome size as a whole) because mutants with deletions in a speci®ed genome can arise after a low-dose mutagenic action, probably not complicated by the heavy damage of the rest of the genome. The latter was the main objection to the conclusion of the study on radiation-induced deletion mutants in Drosophila (Wu et al. 1989), the only work where the association of deletions in satellite DNA with
fitness reduction was demonstrated (Elder and Turner 1995). The parent, in which no deletions of comparable size occur, can be used as a control for even this low-dose mutagenic action.

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Figure 4.—Plot of body mass vs. genome size for R. esculenta frogs, which had metamorphosed in the year of the study (collected in August). ●, Bryansk e Les; ■, Petryatinka; ▲, Sennoe.