

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

Alexander E. Vinogradov* and Alexander T. Chubinishvili†

*Institute of Cytology, Russian Academy of Sciences, St. Petersburg 194064, Russia and

†N. K. Koltsov Institute of Developmental Biology, Moscow 117808, Russia

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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 Vrijen-

hoek 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 Polls-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 $\mu\text{R/hr}$), environs of Petryatinka village (γ -radiation was 60 $\mu\text{R/hr}$), and, near the national nature reserve Bryansky Les, a control point without contamination (γ -radiation was 15 $\mu\text{R/hr}$). 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

Corresponding author: Alexander Vinogradov, Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Ave., 4, St. Petersburg 194064, Russia. E-mail: aevin@mail.cytspb.rssi.ru

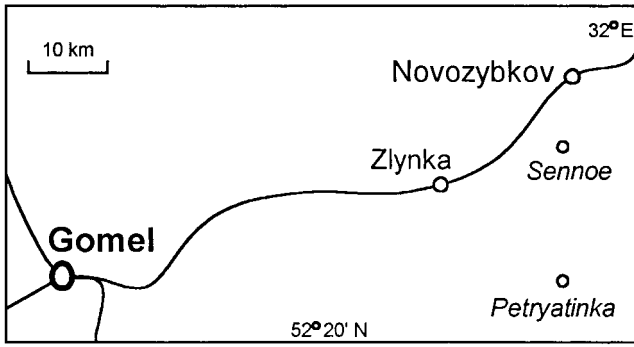


Figure 1.—Map of the radioactively contaminated area where frogs were collected. (For position of Gomel on the large-scale map see *Daily Telegraph World Atlas*, 24, F5.)

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

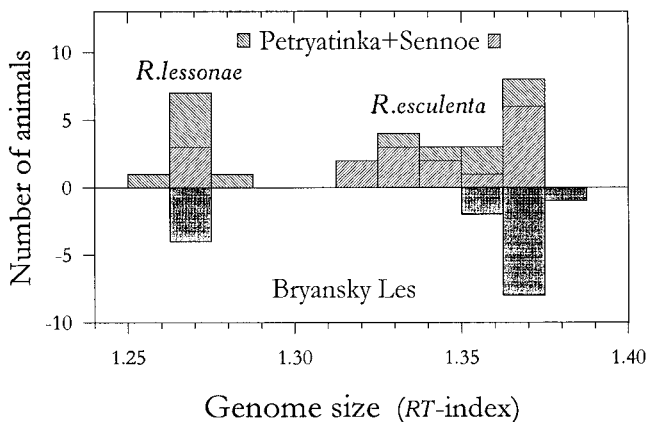


Figure 2.—Population histograms of genome size. Background level of γ -radiation: Sennoe, 220 μ R/hr; Petryatinka, 60 μ R/hr; Bryansky Les, 15 μ R/hr (control point without contamination).

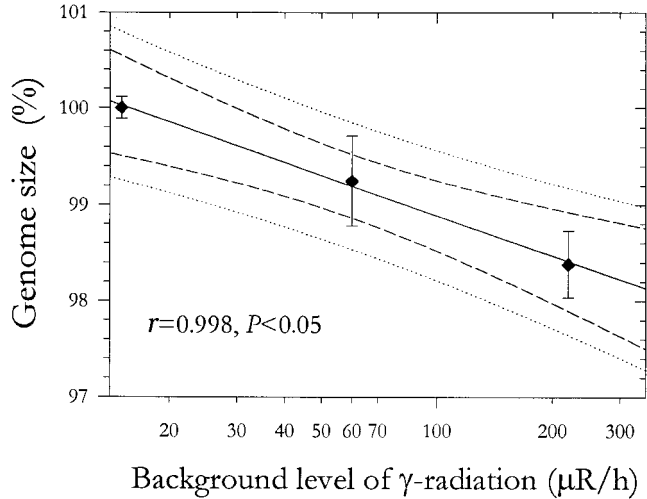


Figure 3.—The relationship between mean genome size (with SE bars) of *R. esculenta* and background level of γ -radiation in the populations studied. The mean genome size of *R. esculenta* from Bryansky Les (control point, 15 μ R/hr) is set at 100%. The linear equation is $Y = 101.7 (\pm 0.15) - 1.4X (\pm 0.08)$, which corresponds to -0.4% of genome size per doubling of γ -radiation level. Dashed lines, confidence limits; dotted lines, prediction limits (both for $P = 0.95$).

(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 (Muller 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, hemiclinal 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

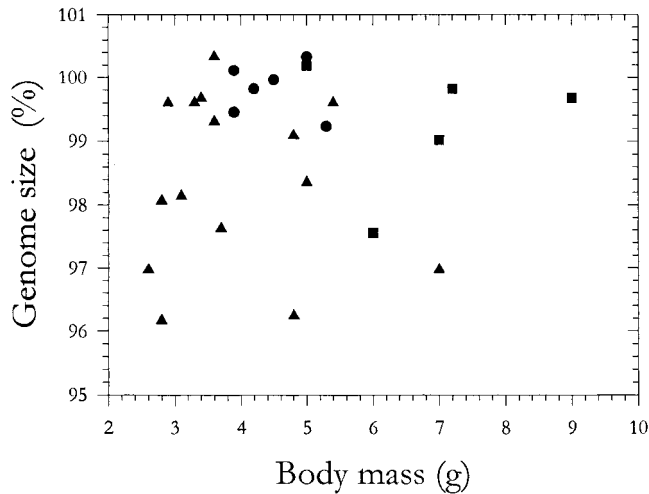


Figure 4.—Plot of body mass vs. genome size for *R. esculenta* froglets, which had metamorphosed in the year of the study (collected in August). ●, Bryansky Les; ■, Petryatinka; ▲, Sennoe.

(probably, of up to 8% in the clonal *ridibunda* genome) were not connected with fitness reduction (because the main adaptive goal of the froglets is to gain weight). Even if the revealed deletions did not involve the coding DNA, the question arises about the biological significance of changes in genome size (or the role of redundant, noncoding DNA). The absence of correlation between genome size and froglets weight suggests the neutralist interpretation for the existence of redundant DNA (Pagel and Johnstone 1992; Charlesworth *et al.* 1994; Elder and Turner 1995). However, the possibility cannot be excluded that the effect of genome reduction would be expressed later, at a low-metabolic stage of overwintering, in accordance with the adaptive (ecophysiological) concept of redundant DNA (Vinogradov 1995, 1998). Note that, because selection pressure is not effective in the sheltered clonal genome, there should be a mutation pressure for the observed reduction in genome size. The same pressure occurs in the case of the Y chromosome, which is usually significantly reduced compared to its counterpart (reviewed by Morell 1994), up to the XO condition in some species. If such reduction is a typical net result of mutation pressure, then accumulation of redundant DNA in larger genomes should be explained by selection pressure.

The hybridogenetic forms could become a suitable object for research on biological significance of individual genomic elements (and of genome size as a whole) because mutants with deletions in a specified 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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LITERATURE CITED

- Berger, L., 1968 Morphology of the F1 generation of various crosses within *Rana esculenta*-complex. *Acta Zool.* **13**: 301–324.
- Charlesworth, B., P. Sniegowski and W. Stephan, 1994 The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**: 215–220.
- Daily Telegraph World Atlas*, 1998 Telegraph Publications, London.
- Elder, J. F., Jr., and B. J. Turner, 1995 Concerted evolution of repetitive DNA sequences in eukaryotes. *Q. Rev. Biol.* **70**: 297–320.
- Giorgi, P. P., 1992 Sex and the male stick insect. *Nature* **357**: 444–445.
- Graf, J. D., and M. Polls-Pelaz, 1989 Evolutionary genetics of the *Rana esculenta* hybrid complex, pp. 289–302 in *Evolution and Ecology of Unisexual Vertebrates*, edited by R. Dawley and J. Bogart. New York State Museum, Albany, NY.
- Leslie, J. F., and R. C. Vrijenhoek, 1978 Genetic dissection of clonally inherited genomes of *Poeciliopsis*. I. Linkage analysis and preliminary assessment of deleterious gene load. *Genetics* **90**: 801–811.
- Leslie, J. F., and R. C. Vrijenhoek, 1980 Consideration of Muller's ratchet mechanism through studies of genetic linkage and genomic compatibilities in clonally reproducing *Poeciliopsis*. *Evolution* **34**: 1105–1115.
- Morell, V., 1994 Rise and fall of the Y chromosome. *Science* **263**: 171–172.
- Muller, H. J., 1914 A gene for the fourth chromosome of *Drosophila*. *J. Exp. Zool.* **17**: 325–336.
- Muller, H. J., 1964 The relation of recombination to mutational advance. *Mutat. Res.* **1**: 2–9.
- Nei, M., 1970 Accumulation of nonfunctional genes on sheltered chromosomes. *Am. Nat.* **104**: 311–322.
- Pagel, M., and R. A. Johnstone, 1992 Variation across species in the size of the nuclear genome supports the junk-DNA explanation for the C-value paradox. *Proc. R. Soc. Lond. Ser. B* **249**: 119–124.
- Quattro, J. M., J. C. Avise and R. C. Vrijenhoek, 1991 Molecular evidence for multiple origins of hybridogenetic fish clones (*Poeciliidae: Poeciliopsis*). *Genetics* **127**: 391–398.
- Rice, W. R., 1994 Degeneration of a nonrecombining chromosome. *Science* **263**: 230–232.
- Turner, H. G., and S. Heppich, 1981 Premeiotic genome exclusion during oogenesis in the common edible frog, *Rana esculenta*. *Naturwissenschaften* **607**: 207–208.
- Uzzell, T., H. Hotz and L. Berger, 1980 Genome exclusion in gametogenesis by an interspecific *Rana* hybrid: evidence from electrophoresis of individual oocytes. *J. Exp. Zool.* **214**: 251–259.
- Vinogradov, A. E., 1995 Nucleotypic effect in homeotherms: body mass-corrected basal metabolic rate of mammals is related to genome size. *Evolution* **49**: 1249–1259.
- Vinogradov, A. E., 1998 Buffering: a possible passive-homeostasis role for redundant DNA. *J. Theor. Biol.* **193**: 197–199.
- Vinogradov, A. E., L. J. Borkin, R. Gunther and J. M. Rosanov, 1990 Genome elimination in diploid and triploid *Rana esculenta* males: cytological evidence from DNA flow cytometry. *Genome* **33**: 619–627.
- Vinogradov, A. E., L. J. Borkin, R. Gunther and J. M. Rosanov, 1991 Two germ cell lineages with genomes of different species in one and the same animal. *Hereditas* **114**: 245–251.
- Wu, C. I., J. R. True and N. Johnson, 1989 Fitness reduction associated with the deletion of a satellite DNA array. *Nature* **341**: 248–251.