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Genetic fallout from Chernobyl

Holographic control of atomic beams

Insect species diversity

A galactic chimney

High levels of genetic change in rodents of Chernobyl

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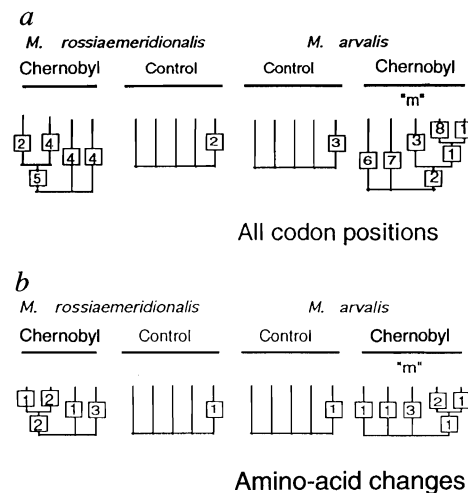
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BASE-PAIR substitution rates for the mitochondrial cytochrome *b* gene of free-living, native populations of voles collected next to reactor 4 at Chernobyl, Ukraine, were estimated by two independent methods to be in excess of 10^{-4} nucleotides per site per generation. These estimates are hundreds of times greater than those typically found in mitochondria of vertebrates, suggesting that the environment resulting from this nuclear power plant disaster is having a measurable genetic impact on the organisms of that region. Despite these DNA changes, vole populations thrive and reproduce in the radioactive regions around the Chernobyl reactor.

The meltdown of reactor 4 on 26 April 1986 at the Chernobyl nuclear power plant was estimated to have released 50–200 million curies of radiation^{1–3} plus a variety of chemical and metal pollutants. To estimate the biological significance of this accident, we have compared the genetic variation of voles living at the Chernobyl site (Chernobyl samples) exposed to this pollution^{3,4} with those collected from a site that received minor amounts of Chernobyl-related pollution (control samples)⁴. The 1,143-base-pair (bp) cytochrome *b* gene was sequenced^{5,6} from two species of voles (*Microtus arvalis*, $n = 5$, Chernobyl sample; $n = 5$, control region; and *M. rossiaemeridionalis*, $n = 4$, Chernobyl sample; $n = 5$ control region). The Chernobyl sample was collected 1 km southwest of reactor 4 in an area formerly known as the Red Forest (UTM 36, 306204E, U 5683716N (coordinates give kilometers east of the 36th TransMercator line and north of equator) 51° 16' 18.4" N, 30° 13' 18.0" E), whereas the control sample was collected from west of Stracholesye, 32 km southeast of reactor 4 (UTM 36, 318000E, U 5661778N; 51° 04' 43.0" N, 30° 24' 06.1" E)⁷. No morphological abnormalities were noted in dissections and specimen preparation of any of these rodents, although eight of 191 specimens from Chernobyl had spleens enlarged to up to six times the normal size⁷.

Using phylogenetic methods (parsimony), the minimum number of nucleotide changes was calculated^{8,9} for the control



populations and the Chernobyl populations. In the resulting trees, significantly more nucleotide substitutions were present in the Chernobyl samples than in the control samples (Fig. 1a and Table 1) (*M. rossiaemeridionalis* $\chi^2_1 = 13.8$, $P < 0.0003$; *M. arvalis* $\chi^2_1 = 20.2$; $P < 0.00001$). The inferred cytochrome *b* amino-acid sequence of each Chernobyl vole was unique, differing from the sequence of every other vole in the sample (Fig. 1b). This level of variation in amino-acid order is unprecedented among the populations of mammals studied so far^{8,9} and it is surprising that this degree of amino-acid evolution is tolerated in the Chernobyl population. In contrast, four of the five control sequences are identical in each species, a level of variation similar to that seen in cytochrome *b* in other rodents^{8,9}.

Estimating the amount of variation present in the Chernobyl population before 1986 as the amount of variation present in the control samples, and subtracting the control total from the total present in the Chernobyl sample, we estimate excess base-pair substitutions as 25(28 – 3) in *M. arvalis* and 17(19 – 2) in *M. rossiaemeridionalis*. Invoking the highly conservative assumption that all nine voles represent independent lineages and estimating the number of generations per year as two (estimated total of 144 generations), the nucleotide-substitution rate for the 1,143-bp gene for 42 mutations is calculated as 2.5×10^{-4} substitutions per nucleotide site per generation. Calculated separately, the rate for *M. arvalis* is 2.7×10^{-4} and for *M. rossiaemeridionalis* is 2.3×10^{-4} substitutions per nucleotide site per generation.

Although our estimates from two separate species at Chernobyl are similar, mutation rates of 10^{-4} are so exceptional (mutation rates for mitochondrial genes are generally 10^{-6} to 10^{-8} per

TABLE 1 Nucleotide substitutions arranged by codon position and effects of amino-acid substitution in two species of voles

Taxon and location	Codon position			Amino-acid effect	
	1st	2nd	3rd	Syn	Non
Chernobyl					
<i>M. rossiaemeridionalis</i>	6	5	8	9	10
<i>M. arvalis</i>	7	5	16	20	8
Total	13	10	24	29	18
Control					
<i>M. rossiaemeridionalis</i>	1	0	1	1	1
<i>M. arvalis</i>	2	0	1	2	1
Total	3	0	2	3	2

Syn, synonymous; Non, non-synonymous.

year)¹⁰⁻¹⁶ that independent confirmation is warranted. In particular, we wanted to investigate the possibility that these increased mutation/substitution rates resulted from immigration of heterogeneous colonists after the meltdown.

We therefore sequenced the cytochrome *b* gene from a Chernobyl-captured female *M. arvalis* which contained five embryos (Fig. 1; 'm' identifies the mother in the tree of *M. arvalis*). To control for polymerase error and to check for mosaicism, DNA was isolated from the mother's liver and heart, and two clones from each tissue were sequenced in both directions. No variation was found among these four clones. For each embryo, two clones were sequenced, and if any site differed from that of the mother (a potential mutation), a third independent amplification of that region from a new aliquot of DNA was sequenced to verify the presence of this base-pair substitution. No variation was detected among different clones from individual embryos. Two embryos had cytochrome *b* genes with nucleotide sequences identical to the mother. Three embryos were distinguished from their mother by one nucleotide change each; two individuals shared a third-position synonymous substitution, and one individual had a first-position non-synonymous substitution. Two independent (twinning, or a single mutation in the germ line before the two oocytes developed could account for the shared mutation) substitutions in 5,715 bp provide a substitution rate estimate of 3.5×10^{-4} , which corroborates the estimates from the populational comparisons above. Although our data are limited to a single female and her embryos, using the highest annual mutation rate normally accepted for the mitochondrial genome¹⁰⁻¹⁶ (1×10^{-6}), the probability of two mutations being observed by chance in 5,715 base pairs is 3.3×10^{-5} . Results from embryos suggest that the high substitution rate within the Chernobyl populations is ongoing, and contradict the hypothesis that the observed populational polymorphisms are the result of immigration. If this elevated mutation rate in cytochrome *b* extends to the entire mitochondrial genome (~17,000 bp), such a rate would translate into 3-5 substitutions per mitochondrial genome per generation. It is probable that such a high mutation rate does not extend to the nuclear genome because such a mutation rate across three billion base pairs would result in 600,000 mutations per gamete.

It must be understood that this increased substitution rate may reflect the presence of mutagens other than, or in addition to, radioactivity, or some synergistic effects of mutagens¹⁷. If these changes were caused solely by ambient levels of radiation, then the substitution rate should decline as the radioactivity decays. Although the level of ambient radiation has declined substantially since the accident¹⁸, our limited analyses of embryos suggest that the substitution rate is still vastly elevated over controls and may be similar to that which led to the current levels of genetic variation in the populations around the reactor. Unlike many of the radioisotopes released at Chernobyl, heavy metals and other mutagenic chemicals can persist indefinitely in the environment. In this and other respects, the environmental pollution resulting from the Chernobyl accident is different from that resulting from nuclear weapons. It does not appear that the biological consequences of the Chernobyl accident can be adequately predicted from results of previous laboratory studies^{19,20} or from the extensive investigations of the effects of Hiroshima and Nagasaki^{21,22}. □

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