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Alpha radiation is a major germ-line mutagen over evolutionary timescales

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ABSTRACT

Question: Is alpha radiation responsible for major changes in genome structure, including karyotypic change and higher ploidy? *In vitro*, high doses of alpha radiation yield many chromosomal changes, while low doses yield mostly point mutations.

Data description: Previously published data on karyotypic variability and the incidence of polyploidy in various animal and plant species.

Search method: In nature, most alpha radiation exposure is caused by radon progeny. Exposure is particularly high below ground, and is also elevated on plant surfaces due to deposition by rain. I look for correlations between the degree of karyotypic variability (or polyploidy) and exposure to alpha radiation.

Conclusions: Both karyotypic variability and the frequency of polyploidy are associated with ecological exposure to alpha radiation. The associations are consistent with observed differences in synonymous substitution rates between species. These results suggest that alpha radiation is the dominant evolutionary mutagen for some species.

Keywords: karyotypic change, mutation, ploidy.

INTRODUCTION

Natural selection operates on the genetic variation provided by mutation. If the spectrum of mutational change is different in two lineages, one might expect to see different patterns of genomic evolution in those lineages.

Alpha radiation of cell cultures at small doses [up to 1.7 mGy (Nagasawa and Little, 2002) or 5 mGy (Huo *et al.*, 2001)] generates a mutational spectrum in which almost all mutations induced by the radiation are small local DNA changes, such as point mutations. Larger doses generate chromosomal mutations such as partial and total gene deletions (Huo *et al.*, 2001; Nagasawa and Little, 2002). Similar changes in spectra are observed for gamma radiation (Schwartz *et al.*, 2000). The general pattern of chromosomal mutations occurring more often with higher exposure is robust, at least *in vitro* (Jostes, 1996; Hei *et al.*, 2004).

Chromosomal mutations are associated with 'direct hits' of nuclei by alpha particles, while point mutations are more common at low doses in the descendents of 'bystander' cells

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(Brenner *et al.*, 2001; Huo *et al.*, 2001; Nagasawa and Little, 2002; Hei *et al.*, 2004). Dose-dependent changes in mutation frequency suggest that point mutations and small deletions may result from single DNA lesions, while large deletions result from pairs of double-strand breaks (Schwartz *et al.*, 2000). Dose rate, cell type, and the genetic locus studied also influence the outcome of radiation exposure (Schwartz *et al.*, 2000). Clustered DNA damage of severity greater than simple double-strand breaks is likely to occur at biologically relevant frequencies with all ionizing radiations (Goodhead, 1994). The mechanism by which alpha radiation induces mutation is not completely understood, and is the subject of ongoing study (Hei *et al.*, 2004). In addition to the bystander effect, radiation can induce genomic instability, in which the offspring of irradiated parents show increased rates of somatic and germ-line mutation (Kadhim *et al.*, 1992; Dubrova, 2003; Niwa, 2003). Instability can be generated in bystander cells (Lorimore *et al.*, 1998; Watson *et al.*, 2000).

If alpha radiation plays a significant role in germ-line mutagenesis over evolutionary timescales, one would expect to see a significantly different pattern of genomic evolution in organisms exposed to high doses of alpha radiation. This pattern would be characterized by a much higher rate of large-scale genomic changes, including chromosomal mutations and changes in karyotype. Synonymous substitution rates would also be somewhat higher in such organisms. Genomic adaptations to mutagenic radiation might also be expected.

Radon is a decay product of uranium, which is widely distributed over the land regions of the earth's crust (Plant and Saunders, 1996). Radon and its decay progeny together constitute the largest natural radiation exposure to humans (United Nations Scientific Committee on the Effects of Atomic Radiation, 2000). Two terrestrial ecological niches are associated with elevated exposure to radon and its decay progeny in animals:

1. *An underground lifestyle*. In soils typical of Ontario and Manitoba, for example, it is estimated that fossorial species receive hundreds of mGy per year to their lungs through inhaling radon and radon progeny (Macdonald and Laverock, 1998). Pocket gophers receive especially high doses, between 530 and 2740 mGy per year (Macdonald and Laverock, 1998).
2. *A grass-eating or leaf-eating lifestyle in areas of relatively high rainfall*. The radon progeny lead-210 and polonium-210 are natural contaminants of grass by atmospheric deposition (Hill, 1960). Leafy plants are also subject to atmospheric deposition (Joshi, 1982; Pietrzak-Flis and Skowronska-Smolak, 1995). Leaves accumulate radionuclides in proportion to their area (Pietrzak-Flis and Skowronska-Smolak, 1995) and the total rainfall (Hill, 1960), and the radionuclide contaminants remain in the leaves (Athalye and Mistry, 1972; Pietrzak-Flis and Skowronska-Smolak, 1995). A lifestyle that involves extended periods of physical contact with mature leaves may also expose animals to leaf-resident radionuclides.

To have germ-line effects, alpha radiation emitters such as polonium-210 must localize to the gonads. The rates of accumulation in the testes and ovaries vary both with the mode of polonium-210 exposure and with the organism studied (Fink, 1950). Polonium-210 accumulates in soft tissues (Hill, 1965), and concentrations in the testes tend to be higher than the body average (Hunt, 1990; Leggett and Eckerman, 2001). Lead-210 accumulates in bones (Hill, 1965), and may support an ongoing polonium-210 exposure. Radon may also be absorbed in fat (Allen *et al.*, 1995).

Estimated levels of alpha radiation exposure and polonium-210 uptake rates to the gonads have been measured in different organisms under various conditions. The general magnitude of these measured quantities suggests that doses to germ-line cells in niches rich

in alpha radiation may be sufficiently large to increase the rate of large-scale genomic changes. Chromosomal mutations (including germ-line changes) attributable to alpha radiation have been observed in some fossorial rodent individuals (Gileva *et al.*, 1996; Gileva and Nokhrin, 2001) and their progeny (Gileva *et al.*, 2000).

To evaluate the hypothesis that alpha radiation influences genomic evolution, I look for patterns in mutation that could be explained on the basis of ecological exposure to alpha radiation. General patterns among large groups will suggest correlations between ecology and mutation. Exceptional species that have unusually high (or low) mutation rates for their groups will be particularly informative. The ways in which such a species differs ecologically from its group will provide strong clues about ecological features associated with mutation.

ANALYSIS

Chromosomal mutation

Chromosomal mutations include fusions, inversions, deletions, and translocations. These kinds of chromosomal mutation occur at different frequencies in different lineages. The kind of change may itself be an indicator of the severity of the underlying DNA damage. For example, under certain experimental conditions, translocations occur in the presence of two double-strand breaks, but not in the presence of one (Richardson and Jasin, 2000). Changes in chromosome numbers (or chromosome arm numbers) per lineage over time (Wilson *et al.*, 1975; Bush *et al.*, 1977) provide a strong indication of rates of karyotypic change, even though they are insensitive to inversions and small deletions. More recent studies have identified changes such as inversions that do not alter the chromosome number (Burt *et al.*, 1999), but such data are currently available for a limited number of lineages only.

Rodents

Rodents as a group have high rates of karyotypic evolution (Bush *et al.*, 1977). Most rodents live in underground burrows. Strongly fossorial rodents such as pocket gophers, mole rats, and tucotucos have extreme chromosomal diversity (Patton, 1972; Bush *et al.*, 1977; Nevo, 1979; Patton and Sherwood, 1983). In contrast, tree squirrels and flying squirrels have a conserved karyotype (Stanyon *et al.*, 2003b; Li *et al.*, 2004) that is closer to the ancestral eutherian karyotype (Li *et al.*, 2004).

Ungulates and horses

The high rate of karyotypic evolution among ungulates and horses (Bush *et al.*, 1977) could be explained by the consumption of large quantities of grass. Camels are an exception to the rule, as they have low karyotypic diversity (Bunch *et al.*, 1985). While camels feed on grass, they live in arid or semi-arid areas in which there is relatively little radionuclide contamination of grass by rain.

Lagomorphs

Lagomorphs have high karyotypic diversity (Bush *et al.*, 1977). However, hares (*Lepus*) are karyotypically conserved, and the ancestral karyotype of the lagomorphs appears to be close to that of the hare (Robinson *et al.*, 1983, 2002). Unlike other lagomorphs, hares do not use a burrow to rear their young.

Primates

The analysis of primates is difficult given the complexity and variety of primate behaviour and diet. Howler monkeys have high levels of karyotypic diversity (de Oliveira *et al.*, 2002), live in tropical regions with high rainfall, and eat substantial quantities of mature leaves (Milton, 1979; de Souza *et al.*, 2002). Geophagy may be a source of ingested radionuclides for howler monkeys (de Souza *et al.*, 2002) and *Callicebus* species (Muller *et al.*, 1997), which are also karyotypically diverse (Stanyon *et al.*, 2003a). Gibbons are tropical animals that also have high levels of karyotypic diversity (Koehler *et al.*, 1995). Gibbons drink by rubbing wet leaves on their bodies and then sucking the water from the fur, which may expose them to radionuclides. On the other hand, baboons have a low level of karyotypic diversity (Wienberg and Stanyon, 1998). Baboons have an unusually efficient mechanism for clearing polonium-210 from their bodies (Fellman *et al.*, 1994; Leggett and Eckerman, 2001), although its impact on the effective radiation dose to the gonads is unclear. Great apes and humans also have a relatively conserved karyotype (Wienberg and Stanyon, 1998). More data on radionuclide exposure to various species are needed before firm conclusions can be drawn about primates.

Insectivores

Most insectivores have some degree of fossoriality, and the rate of karyotypic change for insectivores is moderate (Bush *et al.*, 1977). Shrews (*Soricidae*) are more strongly fossorial, and have wide karyotypic variation (Borgaonkar, 1969).

Marsupials

The marsupials have a moderate degree of karyotypic diversity (Bush *et al.*, 1977), with the highest diversity among kangaroos and wallabies (*Macropodidae*) (Hayman, 1977). Kangaroos and wallabies graze on grass and leaves.

Bats

Bats as a group have a low rate of karyotypic change, but the distribution over different lineages is non-uniform (Baker and Bickham, 1980). The two bat lineages with the highest number of chromosomal changes observed by Baker and Bickham (1980) were *Vampyressa pusilla* and *Uroderma bilobatum*. Both of these tropical species inhabit 'tents' that they build out of large leaves by chewing partial cuts in the leaves (Zortea and De Brito, 2000). Radionuclides may be transferred from leaf surfaces to the skin of bats, and ingested during grooming. Tent construction and salivary deposition (Balasingh *et al.*, 1995) may also contribute to the ingestion of radionuclides.

Frogs

Frogs as a group have a low rate of karyotypic change (Bush *et al.*, 1977), although the *Eleuthorodactylus* species are karyotypically diverse (Bogart and Hedges, 1995). *Eleuthorodactylus* frogs typically live among the ground-level leaf litter (Savage, 2002). This is an unventilated area in which elevated radon concentrations occur [in a similar fashion to the elevation of radon levels in poorly ventilated homes (National Research Council, 1999)]. Frog skin is water-permeable, and extended direct contact with mature leaves would expose leaf-litter frogs to soluble radionuclides such as polonium-210. *Eleutherodactylus* frogs carry their fertilized young in skin pouches.

Lizards

Lizards have a low rate of karyotypic change (Bush *et al.*, 1977), but the *Sceloporus* lizards that burrow (Degenhardt *et al.*, 1996) have high diversity (Hall and Selander, 1973).

Carnivores

Most carnivores, including cats, bears, wolves, dogs, seals, walruses, whales, and sea-lions, have low rates of karyotypic change (Arnason, 1972). Foxes have high diversity (Bush, 1975), which can be explained on the basis of their living in burrows. *Mustelidae* (badgers, otters, skunks, weasels, and relatives) have high karyotypic diversity (Wurster, 1969); most of these animals also burrow.

Birds

Birds are karyotypically conservative (Shields, 1982; Guttenbach *et al.*, 2003). Burrowing owls (*Speotyto cunicularia*, formerly *Athene cunicularia*), however, show a unique karyotype (Schmutz and Moker, 1991) that is sufficiently distinct to define a monotypic genus. Among 136 species in 46 avian genera studied by Shields (1982), there were very few pairs of congeneric species with significant karyotypic differences. The pair with the largest difference was the Eurasian roller and the Indian roller (*Coracias garrulus* and *C. benghalensis*). Eurasian rollers use burrows.

The analysis of chromosomal changes is summarized in Table 1.

Table 1. Rules and outliers in rates of karyotypic change of some terrestrial vertebrates, as explained by exposure to alpha radiation

Group	Rate of karyotypic change (explanation)	Outliers (explanation)
Rodents	High (underground lifestyle)	Tree-squirrels and Flying-squirrels (arborial lifestyle)
Lagomorphs	High (underground lifestyle)	Hares (do not burrow)
Artiodactyls and horses	High (grass eaters)	Camels (arid and semi-arid habitat)
Primates	High (grass and leaf eaters)	Baboons, great apes, humans
Insectivores	Moderate (underground lifestyle)	
Marsupials	Moderate (grass/leaf eaters)	
Carnivores	Low	Foxes, Mustelidae (underground lifestyle)
Frogs	Low	*Eleuthorodactylus* (leaf-litter habitat)
Bats	Low	Tent-making bats (reside in leaf 'tents')
Lizards	Low	*Sceloporus* (underground lifestyle)
Birds	Low	Burrowing owl, Eurasian roller (underground lifestyle)

Note: Rate of karyotypic change measured by r' (Bush *et al.*, 1977), counting total karyotypic changes per lineage per million years: High ($r' > 0.4$), Moderate ($0.4 \geq r' > 0.1$), Low ($0.1 \geq r'$). Values of r' are from Bush *et al.* (1977), except for birds. The bird estimate is based on observations of relatively slow karyotypic evolution in bird species (Shields, 1982; Burt *et al.*, 1999).

Ploidy

Duplication events that increase the ploidy of an organism are likely to be caused by mechanisms unrelated to alpha radiation (Otto and Whitton, 2000). However, one could speculate that there may be selection in favour of polyploids in radiation-rich environments. It is well known that polyploid plants are more resistant to radiation than their diploid relatives (Ichikawa, 1981). Because genes are duplicated, somatic cells would be more robust in the face of radiation damage, relative to a parent diploid species (Ichikawa, 1981). Some diploid plant species are sensitive to small changes in background radiation, and exhibit more somatic mutations in regions of higher natural radioactivity (Mericle and Mericle, 1965). The benefit of polyploidy depends on the timing of the radiation dose relative to the life stage of the cells being irradiated (Conger *et al.*, 1982; von Wangenheim *et al.*, 1995). Since non-sister chromosomes are sometimes used for repairing double-strand DNA breaks (Richardson *et al.*, 1998), additional copies of chromosomes would also aid DNA damage repair. In some human cancer cells, the dose of radiation needed to kill a tumour increases with the number of chromosomes in the tumour (Schwartz *et al.*, 1999).

Conventional genetic arguments would suggest that one of the gene copies would degenerate over time, evolving into a non-functional pseudogene, or acquiring some other function (Otto and Whitton, 2000). However, experimental studies suggest selective pressure for maintaining multiple functional gene copies (Hughes and Hughes, 1993; Otto and Whitton, 2000; Gu *et al.*, 2003). The maintenance of multiple gene copies suggests that the benefit of polyploidy is gene redundancy, consistent with a protective effect against mutation in somatic tissues.

Polyploidy is rare in terrestrial animals (Otto and Whitton, 2000). Otto and Whitton (2000) list 30 polyploid amphibian species (or species groups) and 12 reptile species/groups. Among these, most are either burrowing species or species that estivate underground during periods of water shortage. There are no known polyploid mammals: the one mammal mentioned by Otto and Whitton (2000) was later shown to be diploid (Svartman *et al.*, 2005).

For plant species, atmospheric deposition via rain onto plant surfaces is likely to be the primary form of exposure to alpha radiation (Hill, 1960), and is more important for the above-ground portion of the plant than uptake from the soil (Pietrzak-Flis and Skowronska-Smolak, 1995). If alpha radiation creates a selective advantage for polyploidy, then polyploidy should be associated with high rainfall, and with leafy vegetation having high surface area per unit volume. Table 2 summarizes the data in the literature on plant polyploidy, using any of the

Table 2. Polyploidy in plant groups

Group	Frequency of polyploidy
Ferns	Very high
Grasses	Very high
Monocots	High
Herbaceous dicots	High
Woody dicots	Low
Gymnosperms	Low

Note: Assessment of polyploidy based on Delevoryas (1980), Otto and Whitton (2000), and Levy and Feldman (2002), using any of the several measures of polyploidy proposed in the literature.

several proposed measures of (evolutionarily recent or ancient) polyploidy (Otto and Whitton, 2000). The data are consistent with atmospheric deposition, particularly for grasses, where most (if not all) species are polyploid (Levy and Feldman, 2002). The presence of a protective layer of bark in woody dicots and gymnosperms may shield most living cells from atmospherically deposited radionuclides.

The presence of polyploidy in some fish species (Otto and Whitton, 2000) may reflect the relatively high alpha-emitter content of plankton (Shannon and Cherry, 1967; Cherry *et al.*, 1970), which is comparable to that of grass (Hill, 1960).

There are some interesting unexplained geographic patterns in the distribution of polyploids relative to their diploid progenitors. With a few exceptions, polyploids are more common at higher altitudes and at more polar latitudes (Otto and Whitton, 2000). These patterns could be correlated with patterns of atmospheric deposition of radionuclides (Osburn, 1965; Joshi, 1982). In the northern hemisphere, the ratio of land area to ocean area generally increases with latitude. Terrestrial radon emission is much higher than that from oceans due to the preferential distribution of uranium in the continental crust (Plant and Saunders, 1996). Thus, the atmospheric concentration of radon would generally increase with latitude in the northern hemisphere. Prevailing winds would tend to preserve a latitudinal radon distribution. Since the mean residence time of lead-210 in the atmosphere is 4 weeks, radionuclide deposition would not correlate with local radon emissions (Hill, 1960).

Point mutations

Small doses of alpha radiation tend to generate point mutations (Hei *et al.*, 2004), which is compatible with observed mutation patterns. Alpha radiation should influence the point mutation rate in proportion to dose, over evolutionary timescales.

The alpha-radiation hypothesis would predict significantly higher synonymous substitution rates in groups with higher levels of alpha radiation exposure. Such an increase in synonymous substitution rates has been observed in the mouse (Wu and Li, 1985; Li and Tanimura, 1987; Bulmer *et al.*, 1991; O'hUigin and Li, 1992) and rat (Gibbs *et al.*, 2004) relative to humans. This is consistent with the observation that the evolution of rodent karyotypes has been much faster than that of humans (Stanyon *et al.*, 1999; Bourque *et al.*, 2004). Similarly, the rate of synonymous substitution is higher in Old World monkeys and artiodactyls than in humans (Li and Tanimura, 1987). The synonymous substitution rate in dogs is much less than that in the mouse, and is comparable with that of humans (Kirkness *et al.*, 2003).

Point mutation rates in mice remain higher than those of humans even in laboratory populations not exposed to radiation (Drake *et al.*, 1998). The evolution of genes influencing the mutation rate is governed by the relative costs and benefits of improved replication fidelity (Drake *et al.*, 1998). For mice, the trade-off would favour a higher mutation rate if, over evolutionary time, there was a higher exposure to mutagens.

In plants, the rate of synonymous substitution is particularly high in grasses (Bousquet *et al.*, 1992; Gaut *et al.*, 1992, 1996; Eyre-Walker and Gaut, 1997), and is high in certain other monocot species (Wolfe *et al.*, 1989; Gaut *et al.*, 1992). Within the grasses, woody bamboos have lower synonymous substitution rates than herbaceous bamboos and other grass species (Gaut *et al.*, 1997). Woody angiosperms evolve more slowly at synonymous mitochondrial sites than annuals (Laroche *et al.*, 1997). The synonymous substitution results for animals and plants are consistent with expected alpha radiation exposure, and with the previously noted observations on ploidy and rates of karyotypic change.

Mutation rates are known to be higher in the male germ-line than in the female germ-line for most organisms (Crow, 1997). However, mutations fall into two classes with different sex biases. Mutations that involve gene deletions and other chromosomal mutations occur as often (if not more often) in human females as in males (Crow, 1997). Point mutations, on the other hand, are much more common in males (Crow, 1997). This dichotomy is consistent with alpha-radiation effects, in which chromosomal mutations are more likely to be caused by 'direct hits' of alpha particles, while point mutations are more likely to be caused by bystander effects and/or genomic instability (Brenner *et al.*, 2001; Huo *et al.*, 2001; Nagasawa and Little, 2002; Hei *et al.*, 2004). Direct hits are equally likely in both sexes (assuming similar effective doses to the ovaries and testes). However, mutations due to instability effects become visible only after cell replication, and sperm cells have typically undergone many more replications than ova (Crow, 1997).

Forster *et al.* (2002) have studied the impact of radiation on mitochondrial DNA in a human population residing in an area of high radioactivity. The types of germ-line mutation induced by the radiation closely matched the types of mutation observed over evolutionary time: in both cases, about 95% of mutations are transitions, and the mutations induced by radiation localized to evolutionary mutational hotspots (Forster *et al.*, 2002).

DISCUSSION

Speciation has been correlated with karyotypic change (Bush, 1975; Bush *et al.*, 1977). This correlation could be explained if they had a common cause. Low vagility, small deme size, or small effective population size could promote rapid change via inbreeding and genetic drift (Bush, 1975; Bush *et al.*, 1977).

In evaluating the data on bats, Baker and Bickham (1980) do not find evidence for unusual vagility, deme size, or effective population size in bat species with high measures of karyotypic change. Interestingly, they conclude that the most promising explanation is 'genetic and environmental factors which increase rates of chromosomal mutation'.

An increased rate of chromosomal mutation would also explain the correlation between karyotypic change and speciation. Both processes would be accelerated if there was more karyotypic variation on which selection could operate.

An alternative explanation of the correlation between speciation and karyotypic change depends on the likely unfitness of hybrids between individuals with different karyotypes. Because of this unfitness, karyotypic change may increase the rate at which non-interbreeding subpopulations are created. Group selection for the ability to speciate may then be operating via species selection (Vrba and Gould, 1986). There is little evidence for such a hypothesis. It is hard to imagine a bias in karyotypic evolution that generates selectively favourable mutants for ungulates (but not camels), rodents (but not tree squirrels), tent-making bats (but not other bats), leaf-litter frogs (but not other frogs), rabbits (but not hares), foxes (but not wolves), burrowing birds (but not other birds), and so on.

Vorontsov and Lyapunova (1984) observed that within several rodent groups, chromosomal speciation is markedly elevated in geographic regions of high seismic activity. The reasons for this pattern, observed in multiple locations, remained mysterious (Rosenzweig, 1995). These observations can be explained by the present theory, because uranium concentration and radon emissions are significantly elevated in sheared fault zones (Gunderson, 1991; Plant and Saunders, 1996). Vorontsov and Lyapunova (1984) suggested several possible explanations, including the hypothesis that exposure to radiation is increased by factors 'accompanying the

earthquakes'. However, it does not appear that Vorontsov and Lyapunova were aware of elevated radon levels in sheared fault zones. The association with seismicity can explain some other features of chromosomal variation, such as why some mole rat taxa are chromosomally diverse while others are relatively uniform (Vorontsov and Lyapunova, 1984).

Changes in synonymous substitution rates over time (Bulmer *et al.*, 1991; Burt *et al.*, 1999) could potentially be explained by changes in ecology. The change in rate in the mouse [the rate over the last 12 million years has been much greater than before (Bulmer *et al.*, 1991)] could, in theory, be attributed to the adoption of a fossorial lifestyle by an ancestral rodent species more than 12 million years ago.

Yosida and Parida (1980) suggest that large karyotypic changes in three mammal species could be attributable to high local radiation levels. Differential radiation exposure has been invoked to explain low synonymous substitution rates in whales relative to terrestrial mammals (Schlotterer *et al.*, 1991), although no detailed analysis of this hypothesis is given.

Correlations have been observed between nucleotide substitution rates, body size, metabolic rates, and generation times (Martin and Palumbi, 1993), although there are many exceptions. Such correlations would be expected under an alpha-radiation hypothesis, since bystander effects and genome instability depend to some extent on cell replication after the radiation event.

Long generation times and a slow metabolism, as observed in turtles, might counteract some of the mutagenic effects of alpha radiation and lead to lower mutation rates (Avise *et al.*, 1992; Bowen *et al.*, 1993). Burrowing tortoises (*Testudinae gopherus*) are karyotypically conservative relative to other turtles (Dowler and Bickham, 1982), in contrast to the karyotypic diversity of other burrowing species.

Rodents appear to be tolerant to moderate levels of radiation; in some experiments, irradiated animals appeared fitter than unirradiated animals (Luckey, 1991). This effect is probably due to natural selection favouring characters that are adaptive for underground lifestyles with elevated radiation levels. One should not extrapolate this tolerance to humans, because humans have had a much lower exposure to radiation over evolutionary time.

The advantage of polyploidy in masking mutations to somatic cells is analogous to a similar proposed advantage of diploidy over haploidy (Orr, 1995).

There are additional patterns of karyotypic evolution that are compatible with the alpha-radiation hypothesis, but are not explained by it. Some groups, such as turtles and bats, show a deceleration of the rate of karyotypic change over time (Bickham, 1981). Many taxa display karyotypic orthoselection, in which one kind of karyotypic change predominates (Baker *et al.*, 1985). Additional evolutionary processes, such as selection for particular karyotypic features, may make the overall pattern of karyotypic change more complex.

This article provides additional *in vivo* evidence from natural populations for the bystander effect because, at a coarse level, the mutational spectrum matches that observed *in vitro*. This evidence is valuable since experimental studies of low-dose radiation require very large populations to gain sufficient statistical power (Brenner *et al.*, 2003). This evidence supports arguments (Brenner and Sachs, 2002; Hei *et al.*, 2004) that current estimates of the mutagenic effects of low-level alpha radiation (International Commission on Radiological Protection, 1991; National Council on Radiation Protection, 1993; National Research Council, 1999) may underestimate the true potential for genetic and somatic disease.

The high rate of synonymous substitutions in species exposed to high levels of alpha radiation suggests that alpha radiation is the dominant driver of germ-line mutation in such

species. For these species, the 'molecular clock' may really be an atomic clock, ticking at a rate dependent on the alpha radiation exposure. Phylogenetic dates based on molecular clocks should be adjusted for historical alpha radiation levels at the relevant ecological niche.

Alpha radiation may not have been the dominant mutational driver in species such as humans that are karyotypically conservative and have relatively low rates of synonymous substitution. Nevertheless, radionuclides in human tissues (principally polonium-210) deliver a measurable radiation dose (United Nations Scientific Committee on the Effects of Atomic Radiation, 2000), and may contribute to mutation in humans.

When assessing mutation in modern humans, one must discount the evolutionary evidence for two reasons. First, modern humans may be exposed to alpha emitters that were not significant for ancestral humans, including polonium from cigarette smoke (Little *et al.*, 1965) and urban pollution (James *et al.*, 2004), and radon in poorly ventilated homes (National Research Council, 1999). Second, modern humans live far longer than ancestral humans, substantially beyond reproductive age. Mutations that happen after reproductive age would not be visible in the evolutionary genome.

In humans beyond reproductive age there is increased resorption of bone, particularly in women. Bone contains substantial quantities of lead-210 (Hill, 1965), which has a half-life of 22 years. As bone is depleted, lead-210 and polonium-210 resulting from the decay of lead-210 would be released to other parts of the body. Lead is also released from bone during pregnancy (Gulson *et al.*, 2003). Thus, the skeleton may serve as a reservior of radioactivity that extends the period between exposure to radionuclides and cellular damage.

Alpha radiation from radon and its decay products is responsible for roughly half of all terrestrial radiation exposure to humans (United Nations Scientific Committee on the Effects of Atomic Radiation, 2000). Most exposure is to the lungs, and lung cancer is a significant associated health risk (National Research Council, 1999). Background alpha radiation is estimated to cause 14% of all cases of childhood acute lymphoblastic leukaemia (Committee on Medical Aspects of Radiation in the Environment, 1996). Alpha radiation may be a trigger of autoimmune disease (Weller *et al.*, 1996; Bolviken *et al.*, 2003). For several neurological diseases, radon progeny have been found at elevated levels in the brains of patients (Najbauer *et al.*, 1989; Momcilovic *et al.*, 2001).

Alpha radiation has observable germ-line effects in many species, it can cause genetic and somatic disease, and it may be more harmful than previously thought due to the bystander effect. As a result, renewed attention to the effects of alpha radiation *in vivo* is warranted.

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REFERENCES

Allen, J.E., Henshaw, D.L., Keitch, P.A., Fews, A.P. and Eatough, J.P. 1995. Fat cells in red bone marrow of human rib: their size and spatial distribution with respect to the radon-derived dose to the haemopoietic tissue. *Int. J. Radiat. Biol.*, **68**: 669–678.

Arnason, U. 1972. The role of chromosomal rearrangement in mammalian speciation with special reference to Cetacea and Pinnipedia. *Hereditas*, **70**: 113–118.

Athalye, V. and Mistry, K. 1972. Foliar retention, transport and leaching of polonium-210 and lead-210. *Radiat. Bot.*, **12**: 287.

Avise, J.C., Bowen, B.W., Lamb, T., Meylan, A.B. and Bermingham, E. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines. *Mol. Biol. Evol.*, **9**: 457–473.

Baker, R. and Bickham, J. 1980. Karyotypic evolution in bats – evidence of extensive and conservative chromosomal evolution in closely related taxa. *Syst. Zool.*, **29**: 239–253.

Baker, R.J., Bickham, J.W. and Arnold, M.L. 1985. Chromosomal evolution in *Rhogeessa* (Chiroptera: Vespertilionidae): possible speciation by centric fusions. *Evolution*, **39**: 233–243.

Balasingh, J., Koilraj, J. and Kunz, T. 1995. Tent construction by the short-nosed fruit bat *Cynopterus sphinx* (Chiroptera, Pteropodidae) in southern India. *Ethology*, **100**: 210–229.

Bickham, J.W. 1981. 200 million year old chromosomes: deceleration of the rates of karyotypic evolution in turtles. *Science*, **212**: 1291–1293.

Bogart, J. and Hedges, S. 1995. Rapid chromosome evolution in Jamaican frogs of the genus *Eleutherodactylus* (Leptodactylidae). *J. Zool.*, **235**: 9–31.

Bolviken, B., Celius, E.G., Nilsen, R. and Strand, T. 2003. Radon: a possible risk factor in multiple sclerosis. *Neuroepidemiology*, **22**: 87–94.

Borgaonkar, D.S. 1969. Insectivore cytogenetics. In *Comparative Mammalian Cytogenetics* (K. Benirschke, ed.), pp. 218–246. Berlin: Springer-Verlag.

Bourque, G., Pevzner, P.A. and Tesler, G. 2004. Reconstructing the genomic architecture of ancestral mammals: lessons from human, mouse, and rat genomes. *Genome Res.*, **14**: 507–516.

Bousquet, J., Strauss, S.H., Doerksen, A.H. and Price, R.A. 1992. Extensive variation in evolutionary rate of rbcL gene sequences among seed plants. *Proc. Natl. Acad. Sci. USA*, **89**: 7844–7848.

Bowen, B.W., Nelson, W.S. and Avise, J.C. 1993. A molecular phylogeny for marine turtles: trait mapping, rate assessment, and conservation relevance. *Proc. Natl. Acad. Sci. USA*, **90**: 5574–5577.

Brenner, D.J., Little, J.B. and Sachs, R.K. 2001. The bystander effect in radiation oncogenesis II. A quantitative model. *Radiat. Res.*, **155**: 402–408.

Brenner, D.J., Doll, R., Goodhead, D.T., Hall, E.J., Land, C.E., Little, J.B. *et al.* 2003. Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. *Proc. Natl. Acad. Sci. USA*, **100**: 13761–13766.

Brenner, D.J. and Sachs, R.K. 2002. Do low dose-rate bystander effects influence domestic radon risks? *Int. J. Radiat. Biol.*, **78**: 593–604.

Bulmer, M., Wolfe, K.H. and Sharp, P.M. 1991. Synonymous nucleotide substitution rates in mammalian genes: implications for the molecular clock and the relationship of mammalian orders. *Proc. Natl. Acad. Sci. USA*, **88**: 5974–5978.

Bunch, T., Foote, W. and Maciulis, A. 1985. Chromosome-banding pattern homologies and NORs for the bactrian camel, guanaco, and llama. *J. Hered.*, **76**: 115–118.

Burt, D.W., Bruley, C., Dunn, I.C., Jones, C.T., Ramage, A., Law, A.S. *et al.* 1999. The dynamics of chromosome evolution in birds and mammals. *Nature*, **402**: 411–413.

Bush, G. 1975. Modes of animal speciation. *Annu. Rev. Ecol. Syst.*, **6**: 339–364.

Bush, G.L., Case, S.M., Wilson, A.C. and Patton, J.L. 1977. Rapid speciation and chromosomal evolution in mammals. *Proc. Natl. Acad. Sci. USA*, **74**: 3942–3946.

Cherry, R.D., Shay, M.M. and Shannon, L.V. 1970. Natural alpha-radioactivity concentrations in bone and liver from various animal species. *Nature*, **228**: 1002–1003.

Committee on Medical Aspects of Radiation in the Environment. 1996. Fourth Report. The incidence of cancer and leukaemia in young people in the vicinity of the Sellafield site, West Cumbria: Further studies and an update of the situation since the publication of the report of the Black Advisory Group in 1984. London: Department of Health.

Conger, A., Sparrow, A., Schwemmer, S. and Klug, E. 1982. Relation of nuclear volume and radiosensitivity to ploidy level (haploid to 22-ploid) in higher-plants and a yeast. *Environ. Exp. Bot.*, **22**: 57–74.

Crow, J.F. 1997. The high spontaneous mutation rate: is it a health risk? *Proc. Natl. Acad. Sci. USA*, **94**: 8380–8386.

Degenhardt, W.G., Painter, C.W. and Price, A.H. 1996. *Amphibians and Reptiles of New Mexico*. Albuquerque, NM: University of New Mexico Press.

Delevoryas, T. 1980. Polyploidy in gymnosperms. In Polyploidy (W.H. Lewis, ed.), pp. 215–218. New York: Plenum Press.

de Oliveira, E., Neusser, M., Figueiredo, W., Nagamachi, C., Pieczarka, J., Sbalqueiro, I. *et al.* 2002. The phylogeny of howler monkeys (*Alouatta*, Platyrrhini): reconstruction by multicolor cross-species chromosome painting. *Chromosome Res.*, **10**: 669–683.

de Souza, L., Ferrari, S., da Costa, M. and Kern, D. 2002. Geophagy as a correlate of folivory in red-handed howler monkeys (*Alouatta belzebul*) from eastern Brazilian Amazonia. *J. Chem. Ecol.*, **28**: 1613–1621.

Dowler, R. and Bickham, J. 1982. Chromosomal relationships of the tortoises (family Testudinidae). *Genetica*, **58**: 189–197.

Drake, J.W., Charlesworth, B., Charlesworth, D. and Crow, J.F. 1998. Rates of spontaneous mutation. *Genetics*, **148**: 1667–1686.

Dubrova, Y.E. 2003. Radiation-induced transgenerational instability. *Oncogene*, **22**: 7087–7093.

Eyre-Walker, A. and Gaut, B.S. 1997. Correlated rates of synonymous site evolution across plant genomes. *Mol. Biol. Evol.*, **14**: 455–460.

Fellman, A., Ralston, L., Hickman, D., Ayres, L. and Cohen, N. 1994. Polonium metabolism in adult female baboons. *Radiat. Res.*, **137**: 238–250.

Fink, R.M., ed. 1950. *Biological Studies with Polonium, Radium, and Plutonium*. New York: McGraw-Hill.

Forster, L., Forster, P., Lutz-Bonengel, S., Willkomm, H. and Brinkmann, B. 2002. Natural radioactivity and human mitochondrial DNA mutations. *Proc. Natl. Acad. Sci. USA*, **99**: 13950–13954.

Gaut, B.S., Muse, S.V., Clark, W.D. and Clegg, M.T. 1992. Relative rates of nucleotide substitution at the rbcL locus of monocotyledonous plants. *J. Mol. Evol.*, **35**: 292–303.

Gaut, B.S., Morton, B.R., McCaig, B.C. and Clegg, M.T. 1996. Substitution rate comparisons between grasses and palms: synonymous rate differences at the nuclear gene Adh and parallel rate differences at the plastid gene rbcL. *Proc. Natl. Acad. Sci. USA*, **93**: 10274–10279.

Gaut, B.S., Clark, L.G., Wendel, J.F. and Muse, S.V. 1997. Comparisons of the molecular evolutionary process at rbcL and ndhF in the grass family (Poaceae). *Mol. Biol. Evol.*, **14**: 769–777.

Gibbs, R.A., Weinstock, G.M., Metzker, M.L., Muzny, D.M., Sodergren, E.J., Scherer, S. *et al*. 2004. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature*, **428**: 493–521.

Gileva, E.A. and Nokhrin, D.I. 2001. Chromosomal and ontogenetic instability in sibling species of common vole (*Microtus arvalis* group): comparative aspects. *Zh. Obshch. Biol.*, **62**: 217–225 (in Russian).

Gileva, E.A., Liubashevskii, N.M., Starichenko, V.I., Chibiriak, M.V. and Romanov, G.N. 1996. Hereditary chromosome instability in the common vole (*Microtus arvalis*) from the region of the Kyshtym nuclear accident – fact or hypothesis? *Genetika*, **32**: 114–119 (in Russian).

Gileva, E.A., Nokhrin, D.I. and Starichenko, V.I. 2000. Chromosomal instability in offspring of voles in unfavorable radiation zones. *Genetika*, **36**: 714–717 (in Russian).

Goodhead, D.T. 1994. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. *Int. J. Radiat. Biol.*, **65**: 7–17.

Gu, Z., Steinmetz, L.M., Gu, X., Scharfe, C., Davis, R.W. and Li, W.H. 2003. Role of duplicate genes in genetic robustness against null mutations. *Nature*, **421**: 63–66.

Gulson, B.L., Mizon, K.J., Korsch, M.J., Palmer, J.M. and Donnelly, J.B. 2003. Mobilization of lead from human bone tissue during pregnancy and lactation – a summary of long-term research. *Sci. Total Environ.*, **303**: 79–104.

Gunderson, L. 1991. Radon in sheared igneous and metamorphic rocks. In *US Geological Survey Bulletin*, Vol. 1971 (L. Gunderson and R. Wanty, eds.), pp. 39–50. Washington, DC: USGS.

Guttenbach, M., Nanda, I., Feichtinger, W., Masabanda, J., Griffin, D. and Schmid, M. 2003. Comparative chromosome painting of chicken autosomal paints 1–9 in nine different bird species. *Cytogenet. Genome Res.*, **103**: 173–184.

Hall, W. and Selander, R. 1973. Hybridization of karyotypically differentiated populations in *Sceloporus-grammicus* complex (Iguanidae). *Evolution*, **27**: 226–242.

Hayman, D.L. 1977. Chromosome-number constancy and variation. In The Biology of Marsupials (B. Stonehouse and D. Gilmore, eds.), pp. 27–48. Baltimore, MD: University Park Press.

Hei, T.K., Persaud, R., Zhou, H. and Suzuki, M. 2004. Genotoxicity in the eyes of bystander cells. *Mutat. Res.*, **568**: 111–120.

Hill, C.R. 1960. Lead-210 and polonium-210 in grass. *Nature*, **187**: 211–212.

Hill, C.R. 1965. Polonium-210 in man. *Nature*, **208**: 423–428.

Hughes, M.K. and Hughes, A.L. 1993. Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*. *Mol. Biol. Evol.*, **10**: 1360–1369.

Hunt, V.R. 1990. Polonium-210 measurements in human semen. *Health Phys.*, **58**: 511–514.

Huo, L., Nagasawa, H. and Little, J.B. 2001. HPRT mutants induced in bystander cells by very low fluences of alpha particles result primarily from point mutations. *Radiat. Res.*, **156**: 521–525.

Ichikawa, S. 1981. Responses to ionizing radiation. In *Encyclopedia of Plant Physiology*, Vol. 12A (O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler, eds.), pp. 199–228. New York: Springer-Verlag.

International Commission on Radiological Protection. 1991. *1990 Recommendations of the International Commission on Radiological Protection*. ICRP Publication #60. Philadelphia, PA: Elsevier.

James, P.R., Close, J.J., Keitch, P.A., Allen, J.E., Fews, A.P. and Henshaw, D.L. 2004. Aspects of the geographical variations of naturally occurring 210Pb/210Po in permanent teeth of juveniles in the UK. *Int. J. Radiat. Biol.*, **80**: 199–208.

Joshi, S.R. 1982. Airborne radioactive materials and plants: a review. *Sci. Total Environ.*, **24**: 101–117.

Jostes, R.F. 1996. Genetic, cytogenetic, and carcinogenic effects of radon: a review. *Mutat. Res.*, **340**: 125–139.

Kadhim, M.A., Macdonald, D.A., Goodhead, D.T., Lorimore, S.A., Marsden, S.J. and Wright, E.G. 1992. Transmission of chromosomal instability after plutonium alpha-particle irradiation. *Nature*, **355**: 738–740.

Kirkness, E.F., Bafna, V., Halpern, A.L., Levy, S., Remington, K., Rusch, D.B. *et al.* 2003. The dog genome: survey sequencing and comparative analysis. *Science*, **301**: 1898–1903.

Koehler, U., Bigoni, F., Wienberg, J. and Stanyon, R. 1995. Genomic reorganization in the concolor gibbon (*Hylobates concolor*) revealed by chromosome painting. *Genomics*, **30**: 287–292.

Laroche, J., Li, P., Maggia, L. and Bousquet, J. 1997. Molecular evolution of angiosperm mitochondrial introns and exons. *Proc. Natl. Acad. Sci. USA*, **94**: 5722–5727.

Leggett, R.W. and Eckerman, K.F. 2001. A systemic biokinetic model for polonium. *Sci. Total Environ.*, **275**: 109–125.

Levy, A.A. and Feldman, M. 2002. The impact of polyploidy on grass genome evolution. *Plant Physiol.*, **130**: 1587–1593.

Li, T., O'Brien, P.C., Biltueva, L., Fu, B., Wang, J., Nie, W. *et al.* 2004. Evolution of genome organizations of squirrels (Sciuridae) revealed by cross-species chromosome painting. *Chromosome Res.*, **12**: 317–335.

Li, W.H. and Tanimura, M. 1987. The molecular clock runs more slowly in man than in apes and monkeys. *Nature*, **326**: 93–96.

Little, J., Radford, E., Mccombs, H. and Hunt, V. 1965. Distribution of polonium-210 in pulmonary tissues of cigarette smokers. *New Engl. J. Med.*, **273**: 1343.

Lorimore, S.A., Kadhim, M.A., Pocock, D.A., Papworth, D., Stevens, D.L., Goodhead, D.T. *et al.* 1998. Chromosomal instability in the descendants of unirradiated surviving cells after alpha-particle irradiation. *Proc. Natl. Acad. Sci. USA*, **95**: 5730–5733.

Luckey, T.D. 1991. *Radiation Hormesis*. Boca Raton, FL: CRC Press.

Macdonald, C.R. and Laverock, M.J. 1998. Radiation exposure and dose to small mammals in radon-rich soils. *Arch. Environ. Contam. Toxicol.*, **35**: 109–120.

Martin, A.P. and Palumbi, S.R. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl. Acad. Sci. USA*, **90**: 4087–4091.

Mericle, L.W. and Mericle, R.P. 1965. Biological discrimination of differences in natural background radiation level. *Radiat. Bot.*, **5**: 475–492.

Milton, K. 1979. Factors influencing leaf choice by howler monkeys – test of some hypotheses of food selection by generalist herbivores. *Am. Nat.*, **114**: 362–378.

Momcilovic, B., Alkhatib, H.A., Duerre, J.A., Cooley, M., Long, W.M., Harris, T.R. *et al.* 2001. Environmental lead-210 and bismuth-210 accrue selectively in the brain proteins in Alzheimer disease and brain lipids in Parkinson disease. *Alzheimer Dis. Assoc. Disord.*, **15**: 106–115.

Muller, K., Ahl, C. and Hartmann, G. 1997. Geophagy in masked titi monkeys (*Callicebus personatus melanochir*) in Brazil. *Primates*, **38**: 69–77.

Nagasawa, H. and Little, J.B. 2002. Bystander effect for chromosomal aberrations induced in wild-type and repair deficient CHO cells by low fluences of alpha particles. *Mutat. Res.*, **508**: 121–129.

Najbauer, J., Palffy, G. and Kuntar, L. 1989. Positive autoradiographic findings in brains of 4 MS patients. *Acta Neurol. Scand.*, **79**: 476–481.

National Research Council. 1999. *Health Effects of Exposure to Radon: BEIR VI, Committee on Health Risks of Exposure to Radon*. Washington, DC: National Academy Press.

National Council on Radiation Protection. 1993. *Limitation of Exposure to Ionizing Radiation*. Technical Report #116. Bethesda, MD: NCRP.

Nevo, E. 1979. Adaptive convergence and divergence of subterranean mammals. *Annu. Rev. Ecol. Syst.*, **10**: 269–308.

Niwa, O. 2003. Induced genomic instability in irradiated germ cells and in the offspring; reconciling discrepancies among the human and animal studies. *Oncogene*, **22**: 7078–7086.

O'hUigin, C. and Li, W.H. 1992. The molecular clock ticks regularly in muroid rodents and hamsters. *J. Mol. Evol.*, **35**: 377–384.

Orr, H.A. 1995. Somatic mutation favors the evolution of diploidy. *Genetics*, **139**: 1441–1447.

Osburn, W.S. 1965. Primordial radionuclides: their distribution, movement, and possible effect within terrestrial ecosystems. *Health Phys.*, **11**: 1275–1295.

Otto, S.P. and Whitton, J. 2000. Polyploid incidence and evolution. *Annu. Rev Genet.*, **34**: 401–437.

Patton, J. 1972. Patterns of geographic variation in karyotype in pocket gopher, Thomomys-bottae (Eydoux and Gervais). *Evolution*, **26**: 574–586.

Patton, J. and Sherwood, S. 1983. Chromosome evolution and speciation in rodents. *Annu. Rev. Ecol. Syst.*, **14**: 139–158.

Pietrzak-Flis, Z. and Skowronska-Smolak, M. 1995. Transfer of Pb-210 and Po-210 to plants via root-system and aboveground interception. *Sci. Total Environ.*, 162: 139–147.

Plant, J. and Saunders, A. 1996. The radioactive earth. *Radiat. Protect. Dosimetry*, **68**: 25–36.

Richardson, C. and Jasin, M. 2000. Frequent chromosomal translocations induced by DNA double-strand breaks. *Nature*, **405**: 697–700.

Richardson, C., Moynahan, M.E. and Jasin, M. 1998. Double-strand break repair by inter-chromosomal recombination: suppression of chromosomal translocations. *Genes Dev.*, **12**: 3831–3842.

Robinson, T.J., Elder, F.F. and Chapman, J.A. 1983. Karyotypic conservatism in the genus *Lepus* (order Lagomorpha). *Can. J. Genet. Cytol.*, **25**: 540–544.

Robinson, T.J., Yang, F. and Harrison, W.R. 2002. Chromosome painting refines the history of genome evolution in hares and rabbits (order Lagomorpha). *Cytogenet. Genome Res.*, **96**: 223–227.

Rosenzweig, M. 1995. *Species Diversity in Space and Time*. Cambridge: Cambridge University Press.

Savage, J.M. 2002. *The Amphibians and Reptiles of Costa Rica*. Chicago, IL: University of Chicago Press.

Schlotterer, C., Amos, B. and Tautz, D. 1991. Conservation of polymorphic simple sequence loci in cetacean species. *Nature*, **354**: 63–65.

Schmutz, S. and Moker, J. 1991. A cytogenetic comparison of some North-American owl species. *Genome*, **34**: 714–717.

Schwartz, J.L., Murnane, J. and Weichselbaum, R.R. 1999. The contribution of DNA ploidy to radiation sensitivity in human tumour cell lines. *Br. J. Cancer*, **79**: 744–747.

Schwartz, J.L., Jordan, R., Sun, J., Ma, H. and Hsieb, A.W. 2000. Dose-dependent changes in the spectrum of mutations induced by ionizing radiation. *Radiat. Res.*, **153**: 312–317.

Shannon, L.V. and Cherry, R.D. 1967. Polonium-210 in marine plankton. *Nature*, **216**: 352–353.

Shields, G. 1982. Comparative avian cytogenetics – a review. *Condor*, **84**: 45–58.

Stanyon, R., Yang, F., Cavagna, P., O'Brien, P.C., Bagga, M., Ferguson-Smith, M.A. *et al.* 1999. Reciprocal chromosome painting shows that genomic rearrangement between rat and mouse proceeds ten times faster than between humans and cats. *Cytogenet. Cell Genet.*, **84**: 150–155.

Stanyon, R., Bonvicino, C., Svartman, M. and Seuanez, H. 2003a. Chromosome painting in *Callicebus lugens*, the species with the lowest diploid number ($2n = 16$) known in primates. *Chromosoma*, **112**: 201–206.

Stanyon, R., Stone, G., Garcia, M. and Froenicke, L. 2003b. Reciprocal chromosome painting shows that squirrels, unlike murid rodents, have a highly conserved genome organization. *Genomics*, **82**: 245–249.

Svartman, M., Stone, G. and Stanyon, R. 2005. Molecular cytogenetics discards polyploidy in mammals. *Genomics*, **85**: 425–430.

United Nations Scientific Committee on the Effects of Atomic Radiation. 2000. *Sources and Effects of Ionizing Radiation*. New York: United Nations.

von Wangenheim, K.H., Peterson, H.P. and Schwenke, K. 1995. Review. A major component of radiation action: Interference with intracellular control of differentiation. *Int. J. Radiat. Biol.*, **68**: 369–388.

Vorontsov, N. and Lyapunova, E. 1984. Explosive chromosomal speciation in seismic active regions. *Chromosomes Today*, **8**: 279–294.

Vrba, E. and Gould, S. 1986. The hierarchical expansion of sorting and selection – sorting and selection cannot be equated. *Paleobiology*, **12**: 217–228.

Watson, G.E., Lorimore, S.A., Macdonald, D.A. and Wright, E.G. 2000. Chromosomal instability in unirradiated cells induced *in vivo* by a bystander effect of ionizing radiation. *Cancer Res.*, **60**: 5608–5611.

Weller, R.E., Buschbom, R.L., Dagle, G.E., Park, J.F., Ragan, H.A. and Watson, C.R. 1996. Hypoadrenocorticism in beagles exposed to aerosols of plutonium-238 dioxide by inhalation. *Radiat. Res.*, 146: 688–693.

Wienberg, J. and Stanyon, R. 1998. Comparative chromosome painting of primate genomes. *ILAR J.*, **39**: 77–91.

Wilson, A.C., Bush, G.L., Case, S.M. and King, M.C. 1975. Social structuring of mammalian populations and rate of chromosomal evolution. *Proc. Natl. Acad. Sci. USA*, **72**: 5061–5065.

Wolfe, K., Sharp, P. and Li, W. 1989. Rates of synonymous substitution in plant nuclear genes. *J. Mol. Evol.*, **29**: 208–211.

Wu, C.I. and Li, W.H. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl. Acad. Sci. USA*, **82**: 1741–1745.

Wurster, D.H. 1969. Cytogenetic and phylogenetic studies in Carnivora. In *Comparative Mammalian Cytogenetics* (K. Benirschke, ed.), pp. 310–329. Berlin: Springer-Verlag.

Yosida, T.H. and Parida, B.B. 1980. Karyotype evolution, species differentiation and environmental mutagens. *Proc. Jap. Acad. Sci. B*, **56**: 79–84.

Zortea, M. and De Brito, B. 2000. Tents used by *Vampyressa pusilla* (Chiroptera: Phyllostomidae) in southeastern Brazil. *J. Trop. Ecol.*, **16**: 475–480.