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General Comment

I stand against this PRM. It seems dangerous to me to approve to change the Standards for Protection Against Radiation REGULATIONS based on the hormesis model HYPOTHESIS.

When did that came out that regulations can be based on models that have not been validated (nevermind the tips for submitting an effective PRM)?

I work in a GLP/GMP environment and I a utterly disgusted while reviewing the "scientific basis" supporting this change. The requestors of the change seem to be out of touch with reality or pursue personal goals with no regard to the impact of the change to humans and the natural environment continuity.

The PRM is supported by fallacious statements such as the natural incidence of occult thyroid cancers is approximately 1000 times higher than the highest incidence of reported thyroid cancers in the countries with the greatest fallout from the Chernobyl accident <http://pbadupws.nrc.gov/docs/ML1505/ML15051A503.pdf> p.6

Such statements are NOT supported by facts/evidence! To the contrary:
<http://press.endocrine.org/doi/abs/10.1210/jcem.82.11.4367>

This raises serious doubt about the apparent goal to help prevent unnecessary, radiation-phobia-related deaths,

morbidity, and injuries associated with distrust of radio-medical diagnostics/therapies and from nuclear/radiological emergencies through countering phobia-promoting misinformation spread by alarmists via the news and other media including journal publications.

For any reader of this message, I'd recommend a Levenson Self-Report Psychopathy Scale test:

<http://personality-testing.info/tests/LSRP.php>

Self-Report: 1.7/2.3

Attachments

J Hered-2014-Mousseau-704-9 Genetic and Ecological Studies of Animals in Chernobyl and Fukushima

Genetic and Ecological Studies of Animals in Chernobyl and Fukushima

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Abstract

Recent advances in genetic and ecological studies of wild animal populations in Chernobyl and Fukushima have demonstrated significant genetic, physiological, developmental, and fitness effects stemming from exposure to radioactive contaminants. The few genetic studies that have been conducted in Chernobyl generally show elevated rates of genetic damage and mutation rates. All major taxonomic groups investigated (i.e., birds, bees, butterflies, grasshoppers, dragonflies, spiders, mammals) displayed reduced population sizes in highly radioactive parts of the Chernobyl Exclusion Zone. In Fukushima, population censuses of birds, butterflies, and cicadas suggested that abundances were negatively impacted by exposure to radioactive contaminants, while other groups (e.g., dragonflies, grasshoppers, bees, spiders) showed no significant declines, at least during the first summer following the disaster. Insufficient information exists for groups other than insects and birds to assess effects on life history at this time. The differences observed between Fukushima and Chernobyl may reflect the different times of exposure and the significance of multigenerational mutation accumulation in Chernobyl compared to Fukushima. There was considerable variation among taxa in their apparent sensitivity to radiation and this reflects in part life history, physiology, behavior, and evolutionary history. Interestingly, for birds, population declines in Chernobyl can be predicted by historical mitochondrial DNA base-pair substitution rates that may reflect intrinsic DNA repair ability.

Subject areas: Conservation genetics and biodiversity

Key words: birds, Chernobyl, Fukushima, invertebrates, mutation, radiation

The notion that populations may have evolved adaptive responses to the mutational pressures imposed by chronic, multigenerational exposure to low-dose-rate radioactivity has been a prime motivator for recent studies of populations of plants and animals inhabiting the Chernobyl Exclusion Zone. And the disaster at Fukushima, although ongoing and hugely tragic, provides a unique opportunity to replicate, validate, and certainly test the generality of the findings from studies conducted previously at Chernobyl. The availability of a replicate site with similar characteristics with respect to amount and type of radioactive materials in the environment, as well as significant similarities among species present in both areas, provides for a level of scientific rigor not often available when studying large-scale ecological disasters.

The overarching thesis of the research conducted by our group has been that chronic exposure to radionuclides will result in genetic damage (i.e., single and double strand breaks in DNA) and increased mutation rates (e.g., single nucleotide polymorphism, deletions, insertions, etc.) in both somatic and germ cell lines within individuals. It is expected that some portion of the genetic damage and mutational load will

generate physiological, developmental, and behavioral effects on organisms, and that most if not all expressed variation will be deleterious, more or less. However, to the extent that genetic damage and increased mutational load are expressed as variation in fitness among individuals, effects will be observed at population and community levels and as evolved adaptive responses if genetic variation exists for sensitivity to the effects of radiation.

Genetic Studies in Chernobyl and Fukushima

Among the first rigorous tests for radiation on mutation rates in a wild population at Chernobyl used microsatellite markers to examine *de novo* mutation rates in barn swallows *Hirundo rustica* by comparing microsatellite DNA fingerprints for parents and their offspring (Ellegren et al. 1997). This study found mutation rates to be 2- to 10-fold higher for birds in Chernobyl when compared to control populations in Ukraine and Italy.

A number of similar, although generally less-direct studies, on a wide variety of organisms have subsequently been reported (for reviews, see Zakharov and Krysanov 1996; Møller and Mousseau 2006; Yablokov et al. 2009) and in aggregate, there is little doubt that the contaminants associated with the Chernobyl disaster have generated genetic damage and increased mutation rates, with many studies also finding phenotypic effects that were correlated to the levels of genetic damage reported. In Table 1 of Møller and Mousseau (2006), 33 studies are listed that investigated mutations or cytogenetic effects of increased radiation around Chernobyl compared with control areas in a variety of plant and animal species. There is considerable heterogeneity in the results, with 25 of the studies showing an increase in mutations or cytogenetic abnormalities. Several studies showed an increase in mutation rates for some loci, but not for others. However, many studies were based on small sample sizes, with a resulting low statistical power being unable to show differences of 25% as being statistically significant. Only 4 of these studies investigated germline mutations and these all found significant increases.

Although studies of genetic effects related to the Fukushima disaster have been slow to arrive, a recent seminal study of butterflies exposed to radioactive contaminants associated with the Fukushima disaster also found strong evidence for increased mutation rates as a direct consequence of exposure to radionuclides (Hiyama et al. 2012). The study by Hiyama et al. (2012) was greatly strengthened by laboratory experiments that used both internal and external radiation sources, and these unambiguously validated observations of the elevated mutation rates and phenotypic effects observed in the field (Møller and Mousseau 2013a).

Further support for the hypothesis that low-dose-rate exposures can lead to elevated mutation rates comes from a recent meta-analysis of the effects of naturally occurring radioactive materials on plant and animal populations around the world (Møller and Mousseau 2013b). This study surveyed the results from more than 5000 publications to arrive at 46 studies conducted with sufficient rigor to be included in the meta-analysis. The observed effect sizes for the 66 characters

that could be extracted from these studies are presented in Figure 1. Although many of the individual effects were small and statistically insignificant on their own, overall there were many more that were greater than zero than expected by chance, with an overall average effect size of 0.093 (95% CI = 0.039–0.171) indicating that exposure to naturally occurring radiation accounted for about 1% of the variance in the traits examined. Albeit a small effect, this could still prove significant on an evolutionary time scale. The principal conclusion from this analysis was that there is extensive evidence for small, but significant negative effects of natural variation in background radiation on immune systems, mutation rates, and disease expression across a range of different animals and plants (Møller and Mousseau 2013b). Studies of naturally radioactive areas may also provide opportunities to investigate evolutionary processes of adaptation although no such studies have been conducted to our knowledge.

Developmental Effects: Albinism, Asymmetry, Brain Size, Cataracts, Sperm, and Tumors

There is an increasing array of empirical studies in Chernobyl, and now Fukushima, which document a wide range of physiological, developmental, morphological, and behavioral consequences of exposure to radioactive contaminants. It is presumed that most of these effects have an underlying genetic basis although in some cases, direct toxicity cannot be ruled out. Among the first visible signs of exposure were the appearance of white spots on feathers of birds and perhaps the fur of mammals (i.e., cattle in Fukushima). These “partial albinos” have been well documented for barn swallows in Chernobyl (Ellegren et al. 1997; Møller and Mousseau 2001) and for a number of other bird species as well (Møller, Bonisoli-Alquati, et al. 2013). Barn swallows with aberrant white feathers were first detected in Fukushima in 2012 (Wild Bird Society of Japan 2013) and were observed in increasing frequencies in 2013 and 2014 (Mousseau et al., unpublished data; Wild Bird Society of Japan 2013). Although such partial

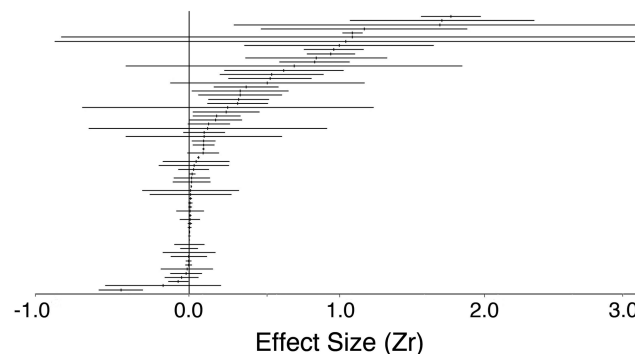


Figure 1. A plot of 66 effect size estimates of the relationship between natural radiation sources and biological response variables of all types. Effect sizes are z -transformed Pearson product-moment correlation coefficient estimates (Z_r) shown with 95% confidence intervals. Redrawn from Møller and Mousseau (2013).

albinos are believed to have reduced probabilities of survival (Ellegren et al. 1997; Møller, Bonisoli-Alquati, et al. 2013), there are sufficient data to suggest that this character can be inherited and may at least in part result from a mutation(s) in the germline, based on parent–offspring resemblance (Ellegren et al. 1997).

Analysis of gametes has served as a proxy for estimates of germline mutation rates for several species of birds in Chernobyl. Møller et al. (2004) reported that the frequency of abnormal sperm in barn swallows was up to 10 times higher for Chernobyl birds as compared to sperm from males living in control areas. They also found that abnormality rates were correlated with reduced levels of antioxidants in the blood, liver, and eggs of these birds, supporting the hypothesis that antioxidants likely play a significant role in protecting DNA from the direct and indirect consequences of exposure to radionuclides. And a more recent analysis of Chernobyl birds found that in 9 out of 10 species examined, sperm abnormality rates were much larger for birds living in Chernobyl than those living in control areas across Europe, with the highest damage levels observed for species with longer sperm (Hermosell et al. 2013), suggesting that sperm abnormalities are likely common for birds living in radioactive areas. Sperm behavior is also negatively affected by radiation exposure history (Møller et al. 2008). Møller et al. (2008) found that sperm swimming ability was negatively related to radiation levels while Bonisoli-Alquati et al. (2011) found that plasma oxidative status could predict sperm performance, further supporting the role antioxidants are known to play in protecting spermatogenesis from the effects of ionizing radiation. Overall, these studies provide convincing evidence that spermatogenesis can be significantly impacted by low-dose radiation and the resulting male infertility may in part explain the smaller population sizes of many species that has been documented for the region (see below).

A recent study of bull sperm and testis from the Fukushima region found no evidence for significant histological changes in the testes or sperm morphology (Yamashiro et al. 2013) although this study was very preliminary with only 2 bulls from a relatively uncontaminated part of Fukushima represented for the analysis of sperm.

Many other cell types and tissues have been shown to be affected by Chernobyl contaminants. Møller, Bonisoli-Alquati, et al. (2013) demonstrated that the frequency of visible tumors on birds was significantly higher in radioactive areas, presumably reflecting elevated mutation rates in somatic tissues. Visible tumor rates in birds from Chernobyl were in excess of 15/1000 birds while tumors have never been observed in Danish populations despite extensive surveys (0/35 000 birds observed) (Møller, Bonisoli-Alquati, et al. 2013). Similarly, the frequency and magnitude of cataract expression in eyes was related to radiation exposure: birds from areas with high background radiation were more likely to display opacities in one or both eyes (Mousseau and Møller 2013). As with radiation-related cataract in humans (e.g., Worgul et al. 2007), there was no relationship with the age of the birds, supporting the hypothesis that radiation was the underlying cause of cataract expression, as were

the levels of genetic damage observed in red blood cells (Bonisoli-Alquati et al. 2010), which are nucleated in birds. Other developmental effects that have been reported include reduced brain size in birds, which is believed to reflect the effects of ionizing radiation on developing neural tissues (Møller et al. 2011), unusual feather shapes and sizes (Møller and Mousseau 2003), and abnormal growth formations on feet and beaks (Møller et al. 2007; Møller, Bonisoli-Alquati, et al. 2013). It remains to be seen whether similar effects will be observed for birds living in the contaminated areas of Fukushima.

Population and Biodiversity Effects of Elevated Mutation Rates

A key issue for evolutionary biologists concerns the fitness consequences of mutation and developmental effects that have been observed for wild populations living in Chernobyl and Fukushima. To this end, we have conducted demographic studies aimed at documenting population sizes, numbers of species (i.e., biodiversity), sex ratios, survival and reproductive rates, and patterns of immigration for animals in both Chernobyl and Fukushima. Because of the highly heterogeneous nature of radionuclide deposition inside the contaminated regions of Chernobyl and Fukushima, it is possible to identify areas that represent the full spectrum of radiation levels, from relatively “clean” uncontaminated habitats all the way to large areas of very high radiation levels, all within short geographical distances. This heterogeneity makes it possible to conduct highly replicated tests for the effects of radiation on biological populations and communities for a single large-scale event. In effect, the distribution of radioactive contaminants, especially in Chernobyl, is more akin to a mosaic or patchwork than diffusion from a point, allowing the uncoupling of radiation levels from distance from the source. It is this lack of geographic structure for radiation levels when combined with multiple tests for radiation effects across multiple habitat types that permits a sensitive analysis of radiation effects independent of other factors.

Abundance and Diversity of Birds, Butterflies, and Other Invertebrates

The first comprehensive surveys of animal abundance and diversity in Chernobyl were conducted by Møller and Mousseau starting in the mid-2000s. The basic sampling protocol was a “massively replicated biotic inventory” design whereby point counts of birds and invertebrates (chiefly, butterflies, dragonflies, bees, grasshoppers and spiders) were conducted at about 300 locations across northern Ukraine and southeastern Belarus in 2006–2008. An identical protocol was used to conduct surveys in Fukushima at 400 distinct locations in 2011–2013. To date, a total of 896 and 1100 biotic inventories have been generated for the Chernobyl and Fukushima regions, respectively. In addition to quantitative estimates of animal abundances and species diversity at each

site, a large number of additional biotic and abiotic factors were measured or estimated including the type of vegetation, the distance to open water, soil type, ambient meteorological conditions, latitude, longitude, elevation, and time of day. All of these variables were included in a multivariate model and used to generate predictions for expected numbers of organisms of each species or group for each location. This model was then used to provide estimates of the variation in numbers explained by radiation independent of all the other potentially contributing factors, in essence, a partial relationship between abundance and radiation. To our knowledge, this approach has not been used in this way before in radioactively contaminated areas although it is used for monitoring bird populations in Europe and North America since the 1960s. It is perhaps the only solution for complex ecological questions of this type, short of large-scale experimental manipulations, which are generally no longer possible for testing the effects of nuclear fission products at a landscape scale. This approach has the added advantage of permitting assessment of ecological effects even in the absence of pre-disaster baseline data.

Contrary to popular notions, the abundance and diversity of forest and grassland birds in Chernobyl were dramatically lower in contaminated areas, showing a dose–response-like relationship, with about one-third as many birds and half as many species present in high contamination areas relative to that predicted by the models (Møller and Mousseau 2007a, 2007b, 2008, 2011a, 2011b). The overall pattern was very similar for birds in Fukushima in July of 2011, with the strength of the negative relationship between abundance and radiation significantly stronger in Fukushima when comparing the 14 bird species that were common to both regions (Møller, Hagiwara, et al. 2012; Møller, Nishiumi, et al. 2013). The observed stronger relationship in Fukushima could reflect the difference between acute and chronic exposures, with Chernobyl bird populations showing a response to 20+ years of selection for resistance, or this could reflect the effects of other radionuclides (e.g., I-131 and Cs-134) that were present at high levels in Fukushima during the spring of 2011 that are no longer present in Chernobyl. Further study will be needed to understand the observed differences. Field studies in Fukushima were also conducted in 2012 and 2013, although results have not yet been published. Continued monitoring of Fukushima populations is an urgent priority for both scientific and management reasons.

In addition to population censuses, there are other lines of evidence supporting the observed declines in population sizes of birds in Chernobyl including changes in adult sex ratios (more males than females) and reductions by half in the number of older birds relative to juveniles and 1-year-olds (Møller, Bonisoli-Alquati, et al. 2012). In addition, there is evidence from analyses of stable isotopes in feathers that the Chernobyl region is acting like a population sink with a higher proportion of immigrants present than in control areas or when compared to birds in historical museum collections from the same area (Møller et al. 2006).

It is interesting to note that as a group, butterflies also showed significant declines with radiation levels in both

Chernobyl and Fukushima (Møller and Mousseau 2009; Møller, Nishiumi, et al. 2013). One might speculate that there is something peculiar about the female ZW sex determination system shared by birds and Lepidoptera (i.e., females are heterogametic) that make these groups particularly vulnerable to mutagenic substances although this idea remains to be tested. One notion might be that because in these groups the heterogametic sex is responsible for egg production, mutational load effects on reproduction stemming from mutation accumulation on the Z chromosome are likely to be expressed immediately following exposure as opposed to species where females are homogametic, as is the case for most sexually reproducing organisms. This might be particularly important given the apparent lack of dosage compensation in birds and Lepidoptera (Parsch and Ellegren 2013). In addition, slightly deleterious mutations may accumulate faster on sex chromosomes than on autosomes (Parsch and Ellegren 2013), and this could be at least in part responsible for the observed greater sensitivity to radiation observed in birds and Lepidoptera.

In most other invertebrate groups examined (e.g., grasshoppers, dragonflies, bees, spiders), population sizes were significantly reduced in areas of high contamination in Chernobyl 20+ years after the disaster while there was no evidence for similar declines in Fukushima and in fact spiders showed significant increases in numbers, at least during the first summer following the disaster (Møller and Mousseau 2009; Møller, Nishiumi, et al. 2013). It has been proposed that such differences in the time course for population effects might reflect the consequences of multigenerational mutation accumulation of recessive deleterious mutations in Chernobyl (Møller and Mousseau 2011a), which is also consistent with the immediate effects on birds and butterflies observed in Fukushima. Alternatively, increases in spider numbers could simply reflect a reduction in predation pressure (e.g., birds).

Predicting Population Responses From Physiology and Historical Base-Pair Substitution Rates

Although there is an overall pattern of decline in animal numbers in direct relation to contamination levels, there is tremendous variability among species in their apparent sensitivity to radionuclides. Many species show no significant relationship between contamination and abundance and a few species even increased with rising radiation levels. Some of the variance in apparent sensitivity can be explained by differences in life history, physiology, and behavior. For example, for birds in Chernobyl, long distance migrants, brightly colored species, and species feeding on invertebrates in the soil showed the strongest negative responses to radiation (Møller and Mousseau 2007b). A recent analysis also found that bird species that used pheomelanin- and carotenoid-based coloration were also particularly sensitive to radionuclides (Galván et al. 2011). These findings point to a critical role played by antioxidants in defending against oxidative stress, and a likely

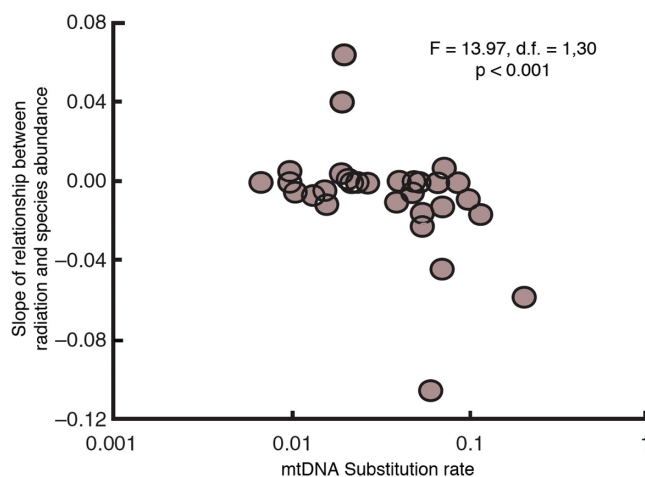


Figure 2. Slope of the relationship between population density of different species of breeding birds and radiation level around Chernobyl in relation to historical mitochondrial substitution rate. Species with the highest historical substitution rates show the greatest declines in numbers if relation to ambient radiation levels. Redrawn from Møller et al. (2010).

physiological trade-off between biochemical precursors that are used for both coloration and as antioxidants. It remains to be seen if interspecific variability in population declines in Fukushima will follow a similar pattern.

Recent evidence suggests that DNA repair may be involved in determining sensitivity to the mutagenic properties of radionuclides. A recent analysis found a significant relationship between the strength of population declines of given species with radiation and historical mtDNA substitution rates for 32 species of birds in Chernobyl (Figure 2; Møller et al. 2010). Species with higher substitution rates showed the greatest declines with radiation levels suggesting that variation in DNA repair capability may be influencing population success although this hypothesis remains to be tested experimentally.

Concluding Remarks

In summary, the radiological disasters at Chernobyl and Fukushima provide a unique opportunity to investigate genetic, ecological, and evolutionary consequences of acute and chronic exposures to mutagenic sources in natural populations at regional and landscape scales. Recent advances suggest many small and large effects on biological systems from molecules to ecosystems that will likely influence ecosystem form and function for decades to centuries to come. The opportunity to compare and contrast organisms from both regions provides for a possible level of scientific rigor (i.e., replication) not previously available for studies of this sort, as well as analysis of the timeframe over which responses may occur and the development of predictive models to aid the management and conservation of biological systems following future nuclear accidents. Given recent advances in molecular genetic technologies, it seems likely that much new knowledge could be gained from a sustained and expansive investment in basic research related to the biological effects of radioactive mutagens within an ecosystem context that extend far beyond the disasters at Fukushima and Chernobyl.

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