American Genetic Association

Journal of Heredity, 2018, 178–187
doi:10.1093/jhered/esx037
Original Article
Advance Access publication 18 April 2017

OXFORD

Original Article

Current Status of the Blue Butterfly in Fukushima Research

Joji M. Otaki and Wataru Taira

From the BCPH Unit of Molecular Physiology, Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Okinawa 903-0213, Japan.

Address correspondence to J. M. Otaki at the address above, or e-mail: otaki@sci.u-ryukyu.ac.jp.

Received December 5, 2016; First decision March 9, 2017; Accepted April 12, 2017.

Corresponding Editor: Tomoko Steen

Abstract

Adverse biological impacts of the Fukushima nuclear accident have been revealed using the pale grass blue butterfly, *Zizeeria maha*, since 2012, which were often considered incompatible with the conventional understanding of radiation biology. This discrepancy likely originates from different system conditions and methodologies. In this article, we first respond to comments from the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) regarding our study; "technical errors" in unit usage and mathematical models noted by UNSCEAR are not errors but reflect our research philosophy not to introduce theoretical assumptions associated with unit conversion and mathematical fit. Second, we review our recent studies to support the original 2012 conclusions. Because the high morphological abnormality rate and small body size detected in Fukushima in 2011 have already ceased, likely through adaptive evolution, their present geographical distributions were investigated throughout Japan. Local populations showing relatively high abnormality rates and small body sizes were rare and basically restricted to Miyagi and its northern populations excluding the Fukushima populations, supporting the causal involvement of the accident. Lastly, we stress the importance of understanding the whole picture of the biological impacts of the Fukushima accident. In addition to the direct radiation impacts, indirect impacts through unknown radiation-associated mechanisms, such as immunological responses to insoluble particulate matter and nutritional deficiencies in plants and animals, would be in effect. Further environmental studies beyond conventional radiation biology and physics are necessary to understand the complex responses of organisms, including humans, to the Fukushima nuclear accident.

Subject area: Conservation genetics and biodiversity
Key words: environmental pollution, Fukushima nuclear accident, geographical distribution, indirect pathway, pale grass blue butterfly, UNSCEAR

The effects of naturally occurring background radiation on biological entities under field conditions have been known for almost a century (Møller and Mousseau 2013a, 2013b), but the biological impacts of the Chernobyl accident have been debated intensely and are indeed poorly understood (Møller and Mousseau 2006). Scientific understanding of the biological consequences of the Fukushima nuclear accident is thus crucial to resolve the issue of the effects of radiation and nuclear pollution under field conditions.

More than 6 years have already passed since the Fukushima nuclear accident in March 2011. Since the first publication on its biological effects in the pale grass blue butterfly, *Zizeeria maha* (Hiyama et al. 2012a), our team has received many positive and negative comments from scientists and the public (e.g., Callaway 2013; Møller and Mousseau 2013b). We have responded to major questions and comments in previous articles (Hiyama et al. 2013; Taira et al. 2014, 2015a). Importantly, our studies have focused on morphological abnormalities, deaths, and other physiological changes using field surveys and laboratory experiments (Hiyama et al. 2012a, 2013, 2015; Nohara et al. 2014a, 2014b, 2017; Taira et al. 2014, 2015; Otaki 2016). As a result, we have presented systematic evidence that morphological abnormalities, deaths, and other physiological changes in this butterfly species in the contaminated areas were caused by the Fukushima nuclear accident. Such evidence must be multifaceted, and further studies should support or correct our findings.

In addition to the accumulation of scientific evidence, a philosophical discussion on how one can accept the causality of the accident (or a release of pollutants in general) on the adverse biological consequences is to be clearly presented. We have proposed 6 criteria called "the postulates of pollutant-induced biological impacts" in analogy with Koch's postulates of infectious diseases (Taira et al. 2014). When all 6 criteria are met, the environmental pollutant (or group of pollutants) in question should be considered a causal factor. The 6 criteria are 1) a spatial relationship between the abnormalities in an organism and the distance from the source of the pollutant in question, 2) a temporal relationship between the abnormalities in an organism and the time of release of the pollutant in question, 3) detection of the pollutant in organisms, that is, demonstration of direct exposure to the pollutant that could cause abnormalities in organisms in the field, 4) phenotypic variability or a spectrum of abnormalities in organisms, 5) experimental reproduction of abnormalities that are seen in the field by external exposure, and 6) experimental reproduction of abnormalities that are seen in the field by internal exposure. Among the 6 criteria, reasonable dose–dependent relationships are expected. Although there are some points that must be improved in the future, our studies on the blue butterfly largely satisfy all 6 criteria.

Meanwhile, independent studies have gradually accumulated that suggest that adverse biological effects of the Fukushima nuclear accident are likely to have occurred in many different animals and plants. These studies have focused on birds and arthropods, showing a decrease in birds, butterflies, and other taxa at the population level (Møller et al. 2012, 2013; Mousseau and Møller 2014; Garnier-Laplace et al. 2015); gall-forming aphids, showing severe morphological abnormalities in field samples (Akimoto 2014); the Japanese monkey, showing a low number of white blood cells (Hayama et al. 2013; Ochiai et al. 2014); the barn swallow, showing a decrease in number (Bonisoli-Alquati et al. 2015); the goshawk, showing reproductive difficulty (Murase et al. 2015); and intertidal biota, showing a drastic decrease in number among several species only in the vicinity of the Fukushima Daiichi Nuclear Power Plant (Horiguchi et al. 2016). Studies on plants address the rice plant, showing aberrant leaf development (Hayashi et al. 2014), and Japanese fir trees, showing morphological defects in the leader shoots (Watanabe et al. 2015).

After the Fukushima nuclear accident, the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) published a report that contains summaries and evaluations of published articles on the biological effects of the Fukushima nuclear accident from UNSCEAR's viewpoint (UNSCEAR 2013). Subsequently, additional reports were published in 2015 (UNSCEAR 2015) and in 2016 (UNSCEAR 2016). The publication of the report in 2016 soon after that in 2015 likely reflects a rapid increase in new articles on this topic. Our studies on butterflies were discussed in these reports.

Here, this article consists of 3 parts that more or less correspond to the past, present, and future states of Fukushima research regarding the blue butterfly. These 3 topics are not tightly interrelated, but they represent the important topics to be discussed at present. On the other hand, the important aspects that are associated with ingestion and transgenerational effects are discussed only briefly here because these topics have been covered elsewhere (Taira et al. 2014, 2015a). The issue of sensitivity variation is not discussed here at all because it has been covered elsewhere (Fukunaga and Yokoya 2016; Otaki 2016).

In this article, we first briefly respond to comments on our studies from UNSCEAR reports. It is important to clarify our standpoint in response to these comments because they likely represent the important criticisms from radiation physicists and radiation biologists in general. We also discuss some related issues arising from UNSCEAR reports. Second, we briefly summarize our current field studies on the geographical distributions of the morphological abnormality rate (often simply called the abnormality rate) and body size of this butterfly. These studies support the previous conclusions that the high abnormality rate and small body size detected in Fukushima in 2011 are consequences of the nuclear accident. Third, we discuss the importance of considering indirect pathways that are associated with nuclear pollution to develop the full picture of the biological effects of the Fukushima nuclear accident.

Response to UNSCEAR (2013, 2015, 2016)

UNSCEAR 2013 Report on Butterfly Studies

In Hiyama et al. (2012a), we performed the field sampling of butterflies from the polluted areas in May and September 2012. We found some correlations in morphological abnormality rate, forewing size, and eclosion time with ground radiation dose or distance from the Fukushima Daiichi Nuclear Power Plant. In addition to the examination of the field samples (defined as the P generation), we obtained offspring generations (defined as the F_1 and F_2 generations) from female butterflies from various localities with different contamination levels and observed higher abnormality rates in the F_1 and F_2 generations than in the P generation, suggesting that the biological effects have been transgenerational (genetic or epigenetic) and that the abnormality rates in the field samples were likely underestimated. Comparison of the P and F_1 results between the May and September samples revealed the accumulation of genetic (or epigenetic) damage over generations in the field. Furthermore, we performed external and internal exposure experiments using butterflies from a minimally contaminated area, Okinawa. Especially important is the internal exposure experiment in which wild leaves of the host plant *Oxalis corniculata* were collected from the contaminated localities and were given to larvae from Okinawa. These individuals showed high mortality and severe morphological abnormalities.

The study above was mentioned in Paragraph 197 (UNSCEAR 2013) as follows. In this paragraph, M22 and M23 refer to Møller et al. (2012) and Møller et al. (2013), respectively, and H6 refers to our original study, Hiyama et al. (2012a).

> 197. A few field studies have reported effects in areas affected by FDNPS releases, such as decreases in bird and insect populations [M22, M23] and morphological and genetic disturbances in butterflies [H6]. The relationship between exposure and effect has not been unequivocally

> established in these studies. Furthermore, the observations are not consistent with the Committee's assessment and suggest that further analysis is needed to establish whether radiation exposure was an important factor, among many others, including the impact of the tsunami itself, in causing the environmental effects observed.

We agree that the original study (Hiyama et al. 2012a) should be confirmed by additional studies because the results are not compatible with the conventional understanding of UNSCEAR and radiation biology. We can readily exclude the possibility of tsunami, as stated explicitly in Hiyama et al. (2013), because we collected butterflies only from cities that were not reached by the tsunami and where people normally live. Moreover, our exposure experiments exclude the possibility of confounding factors experimentally.

We believe that our results and the conventional understanding of radiology are both correct under the conditions of each experiment. These are probably two different sides of the truth based on different system conditions and methodologies. However, in regard to Fukushima, our results are more relevant because our focus is directly placed on Fukushima itself. Radiological studies under laboratory conditions may be irrelevant to Fukushima because their conditions are likely very different from Fukushima.

It is important to fill the gap between the two sides of the truth mechanistically. Under field conditions, in addition to the direct irradiation, indirect radiation-associated pathways that have not yet been identified could simultaneously contribute to the biological effects. This possibility is discussed later.

UNSCEAR 2015 Report on Butterfly Studies

Comments from UNSCEAR (2015) on our original and subsequent studies are found in Paragraph 83 as follows. In this paragraph, H7, N4, T1, and H6 refer to Hiyama et al. (2013), Nohara et al. (2014a), Taira et al. (2014), and Hiyama et al. (2012a), respectively.

> 83. Several papers [H7, N4, T1] provided a comprehensive defence of an earlier publication cited in the 2013 Fukushima report concerning the impacts of radionuclide releases on the Pale Grass Blue Butterfly (*Pseudozizeeria maha*) [H6]. The authors provided an in-depth description of the methods applied and more detailed data analyses. Furthermore, one particular study [N4] augmented the general findings by studying the impact of ingestion of leaves on the larvae of the aforementioned butterfly species. The authors of this suite of publications maintained that exposures due to releases from the FDNPS accident would have led to mortality and abnormalities in the studied butterfly species, that mutations would have been passed on to the progeny and that populations would have decreased considerably in areas close to FDNPS. They further rejected the possibility of confounding factors such as the impact of the tsunami itself. Whilst noting some technical errors, where doses were wrongly specified in units of becquerels and reference was made to dose–response models that were inappropriate for the end points being studied in some of these publications (e.g. Nohara et al. [N4]), the observations indicating increases in particular effects that were correlated with indicators of radiation dose under field-relevant conditions merit further investigation. The publications cannot be easily dismissed nor, accepting the integrity of the datasets, can the results be convincingly explained using existing understanding of radiation effects on environmental systems.

Additionally, in Paragraph 87, 8 articles from our group and other groups are summarized in a table. These articles report "population-level effects on invertebrates and birds that were different from what could be inferred from the Committee's assessment" (UNSCEAR 2015). In Paragraph 92, some publications "that have been judged to make a significant contribution to addressing the research needs identified in the 2013 Fukushima report" (UNSCEAR 2015) are summarized in another table. We appreciate UNSCEAR's open-minded attitudes toward these publications.

Paragraph 83 above summarizes our studies well. As stated, the localities from which we collected butterflies have never been affected by a tsunami. The fact that our feeding experiment (the internal exposure experiment) successfully reproduced high mortality and abnormality rates in a dose-dependent manner confirms that contaminants eaten together with the host plant leaves are responsible for the high mortality and abnormality rates.

The critical comments in Paragraph 83 are technical ones. UNSCEAR (2015) stated that we wrongly used the radioactivity of radiation source, that is, becquerel (Bq), to describe the absorbed radiation doses (Gy). However, this is not an error but rather reflects our research philosophy. The gray (Gy) or sievert (Sv) cannot be used accurately, at least in our systems, because to obtain these values, calculations are required based on many unreliable assumptions. We thus used the original values (Bq) obtained from a germanium semiconductor device. Likewise, to report the contamination levels of the ground, we used the original values (μSv/h) obtained from a NaI(Tl) scintillation survey meter, although we understand that Sv is used only for human doses. This way, we avoided introducing incorrect assumptions into the calculations.

Although we appreciate the importance of dosimetry, we stressed in Hiyama et al. (2012a) whether any systematic adverse changes are detectable and how they are related to the accident using original radiation doses and distance from the Fukushima Daiichi Nuclear Power Plant as pollution indicators. Thus, further studies are necessary to understand the mechanistic pathways. For example, we are sure that the larvae received intense β rays in addition to γ rays because the larvae live very close to the ground. However, this issue has not yet been adequately addressed. A full consideration of β-ray doses may improve our correlation coefficients (Endo et al. 2014).

Similarly, we analyzed mathematical model fits without assuming the model equations a priori. We used all the available models in our computer software, and the models with the highest R^2 or with the lowest information criterion, such as Akaike's information criterion, were considered the best-fit models (Nohara et al. 2014a). Again, this is our effort to not introduce incorrect assumptions. Immediately after the publication of UNSCEAR (2015), we published more detailed descriptions of the mathematical model fits that cover our data from low to high levels of contamination (Taira et al. 2015a). Among the 12 models tested, a sigmoidal Weibull function model and a power function model were considered to be those with the best fits. Because Weibull functions have been used in the analysis of system failure in general, our data fitting a Weibull function may be relevant in analyzing the dose–response curve of abnormality rates.

UNSCEAR 2016 Report on Butterfly Studies

Although our articles are cited in UNSCEAR (2016), no substantial comments are provided. This is likely because there are already many additional reports that suggest the adverse biological impacts of the Fukushima nuclear accident in various organisms. Again, UNSCEAR (2016) maintains that these results are not consistent with the current understanding of radiation biology but encourages further studies.

Other Issues in UNSCEAR Reports

Scientific Name of the Pale Grass Blue Butterfly

In Paragraph 83, UNSCEAR (2015) did not use *Z. maha* but used *Pseudozizeeria maha* as the scientific name of the pale grass blue butterfly, despite our use of *Z. maha*. To be sure, even among lepidopterologists, both *Z. maha* and *P. maha* are sometimes used interchangeably. However, this could be a source of unnecessary confusion in the literature. We have evidence that *Z. maha* is the correct scientific name taxonomically and phylogenetically, and it is the formal name recognized by Japanese lepidopterologists in the latest butterfly archives, that is, Shirôzu (2006), Yago and Odagiri (2007), and Inomata et al. (2010). We will be able to discuss this point more concretely in the future.

Reference Animals Versus Indicator Species

UNSCEAR (2008, 2013) followed the reference animals and plants (RAPs) concept endorsed by the International Commission on Radiological Protection (ICRP) (ICRP 2007, 2008). Among reference animals, a representative "above ground invertebrate" to be studied for environmental protection is bees. The RAPs concept pertains to dosimetry and is thus different from the concept of indicator species, which is useful in field studies. Nonetheless, reference animals could influence the decisions of researchers regarding which animals are to be employed as indicator species in field studies.

Although we generally think that diverse animals and plants should be studied in the field, some are more suitable as indicator species than others. We here state some concerns regarding the use of bees as indicator species. First, some bees (i.e., honey bees) are domesticated animals, and they likely represent a majority of the individuals in local bee populations at least in some areas. Environmental monitoring using domesticated animals may not be encouraged; honey bees are someone's property and not entirely wild entities. Furthermore, bees are dangerous animals for people to collect and are not particularly conspicuous from a distance in the wild.

In contrast, butterflies are wild entities and not dangerous to humans at all. Moreover, butterflies are conspicuous from a distance in the wild and are extremely well-studied from a natural history perspective. Overall, butterflies can likely serve as an indicator of environmental changes as well as (or better than) bees. Indeed, the effects of climate warming on the northward range expansion of organisms were first demonstrated in butterflies (Parmesan et al. 1999; Parmesan 2003).

To be sure, we do not intend to undermine the functional advantages of bees in other studies. Bees are often used in behavioral and neurobiological studies (Eisenhardt 2014) and in environmental studies on pesticides (Sánchez-Bayo et al. 2016), and bees may have more genetic and genomic information than butterflies (Honey Bee Genome Sequencing Consortium 2006; Elsik et al. 2014). However, molecular information on butterfly genomes has rapidly accumulated from several species (Zhan et al. 2011; Heliconius Genome Consortium 2012; Ahola et al. 2014; Kunte et al. 2014; Cong et al. 2015a, 2015b, 2016a, 2016b; Li et al. 2015; Nishikawa et al. 2015). Although the establishment of pure lines (inbred strains) is difficult in *Z. maha* due to inbreeding depression (Iwata et al., 2013), we are currently working on the *Z. maha* genome project, and its genomic sequences will be available soon.

It is also to be noted that the RAPs concept does not force researchers to exclusively study the proposed organisms. The reference bee is not even a real bee but an "idealized" bee for the purpose of dosimetric reference to show a range of reference dose levels called derived consideration reference levels (ICRP 2008). This type of radiological approach is based on the assumptions that direct irradiation is the sole mechanism that disrupts biological entities under wild conditions and that simple dosimetry reveals biological safety levels. It is also often assumed that effects are exclusively directed toward DNA damage.

Although these assumptions are important in speculating on direct irradiation effects, there are problems associated with this standpoint when investigating the biological effects of a nuclear accident in the wild because in addition to the direct DNA damage, many unknown physiological processes likely occur. Nuclear pollution likely triggers complex biological and ecological responses that cannot be reduced to the effect of direct irradiation alone (see below).

Geographical Distributions and Temporal Changes of Abnormalities

Basic Information on the Morphological Abnormality Rate

The basis of biology of the pale grass blue butterfly *Z. maha* (i.e., *Zizeeria* biology) is the establishment of a rearing system (Hiyama et al. 2010) and field and experimental studies (Otaki et al. 2010; Hiyama et al. 2012b). Populations of this butterfly in the margin of its northern range in Aomori Prefecture (Figure 1) were studied from the viewpoint of evolutionary developmental biology (Buckley et al. 2010; Otaki et al. 2010; Hiyama et al. 2012b). Based on these studies, it has become practical to employ this butterfly as an indicator species that is also amenable to experimental manipulations.

It has also become clear that the health status of local populations can be revealed by examining the morphological abnormalities of this butterfly species simply because abnormalities can be considered manifestations of disease. We identify an abnormality only when it was obviously different from its normal counterpart. Ambiguous ones were considered normal. Various kinds of abnormalities were detected, including the absence of leg segments, the deformation of antennae, eye deformation, thoracic tumors, wing deformation, and wing color pattern changes (for more details, see Hiyama et al. 2012a, 2013; Taira et al. 2014). Abnormalities are often found on either the right or left side. This unilateral asymmetric nature is helpful in identifying abnormalities because insect morphology is basically bilaterally symmetric with minor exceptions. Wing color pattern changes are also easy to pinpoint in this species because black spots are positioned in an organized manner against a light-colored background (Hiyama et al. 2013; Iwata et al. 2013).

Seasonal effects on the abnormality rate have not been examined because the abnormality dynamics over 3 years (2011–2013) (Hiyama et al. 2015) do not seem to suggest such effects. Seasonal effects on body size were examined in Taira et al. (2015b), but the effects were irrelevant to our discussion here (see below) because any body size

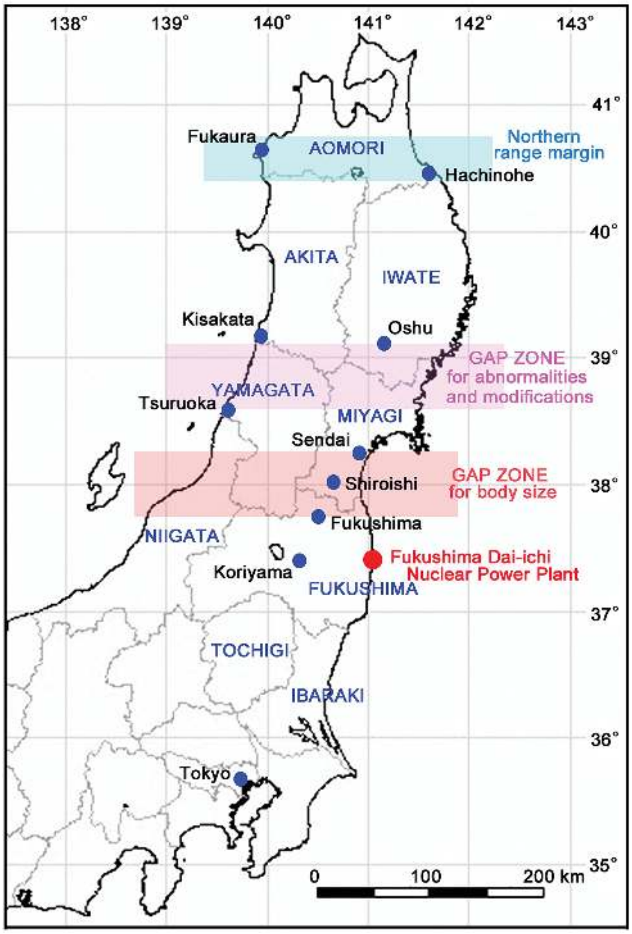

Figure 1. Geographical gaps in the distributions of morphological abnormality rate, wing color pattern modification rate, and body size (forewing size) of the pale grass blue butterfly in Japan. A gap for body size is located at the boundary between Miyagi and Fukushima Prefectures (Taira et al. 2015b). A gap for morphological abnormalities and wing color pattern modifications is located at the boundary between Iwate and Miyagi Prefectures (Hiyama et al. 2017). The northern range margin is located in Aomori Prefecture (Otaki et al. 2010). Prefectural boundaries are drawn with fine solid lines, and prefectural names are shown in uppercase letters. This map was produced by KenMap v9.11 (2015).

comparison was made between locality groups within a given sampling period. In contrast, differences in the number of generations per year in different localities in Japan may affect the speed of adaptive evolution (see below), although we have not examined this possibility.

Rationale for Examining the Morphological Abnormality Rate

Thanks to a previous study on the northern range margins (Otaki et al. 2010), it becomes interesting to know the state of this butterfly in regions other than the northern range margins in Japan for comparison. From the standpoint of environmental protection, it is important to start monitoring indicator species even before an accident occurs. This is to prepare for future accidents and to establish a reference dataset of the morphological abnormality rate under normal conditions. This data set will represent the preaccidental state in the future. One might think that future accidents are unlikely, but a study predicts that such accidents are not unlikely (Wheatley et al. 2017), and another study estimated that severe nuclear accidents such as Chernobyl and Fukushima have large-scale global impacts (Lelieveld et al. 2012).

There is another important reason to now monitor this species throughout Japan. Because the high abnormality rates that were observed in Fukushima in 2011 have now ceased (Hiyama et al. 2015), the current state of this butterfly is likely to be similar to its state before the accident. If so, we now have an opportunity to understand its preaccidental state. Considering that the lack of past data was criticized (Hiyama et al. 2013), the present monitoring attempt is important.

Distribution of the Abnormality Rate in Japan

We executed a survey in northeastern Japan in 2014 to construct a geographical distribution map of the morphological abnormality rate (Hiyama et al. 2017). In this study, we found that the mean abnormality rate was 4.4% throughout the northeastern Japan localities that were surveyed in 2014, but when the geographical distribution data were scanned from south to north, a transition boundary where the abnormality rate was more than the mean + 2 SD (standard deviation) was found (Hiyama et al. 2017). That is, there is a possible gap that divides the northern populations from the rest. The mean abnormality rate excluding the northern populations was 3.0%. This gap is located at approximately 39°N, more than 100 km north of the Fukushima Daiichi Nuclear Power Plant (Figure 1). This gap roughly corresponds to the boundary between Miyagi and Iwate Prefectures. From this survey, we conclude that the high abnormality rate observed in Fukushima in 2011 was highly likely caused by the Fukushima nuclear accident.

We also simultaneously examined the wing color pattern modification rate. In our studies, modifications are defined as phenotypically plastic changes and are fundamentally different from abnormalities (Buckley et al. 2010; Otaki et al. 2010; Hiyama et al. 2012b). The modification rate did not increase in Fukushima in 2012 and is thus not relevant to the biological impacts of the accident. The modification rate was detectable throughout the Kanto-Tohoku district; it was 2.7% on average in 2014. This means that color pattern modifications (as well as morphological abnormalities) spontaneously occur at low levels regardless of the nuclear accident (and thus even before the accident). When the geographical distribution data were scanned from south to north, a transition boundary where the modification rate was more than the mean + 2 SD was found as a gap (Hiyama et al. 2017). Excluding the northern populations, it was 1.1% on average. It is interesting that the gap for the modification rate coincides with that for the abnormality rate (Figure 1). It is likely that both the modification gap and the abnormality gap existed even before the nuclear accident.

We further carried out a survey in southwestern Japan in 2015 that included the localities of 3 nuclear power plants that were planned to resume operations: Sendai (Kagoshima), Ikata (Ehime), and Takahama (Fukui) Nuclear Power Plants. This southwestern study will be published elsewhere in the future.

Body Size Distribution in Japan

We have also studied the geographical distribution of body size (represented by forewing size) throughout Japan. This is because we detected a small forewing size in the butterfly in the spring of 2011; Fukushima samples were smaller than northern and southern samples (Hiyama et al. 2012a, 2013). It is to be noted that Fukushima samples were significantly smaller than northern Shiroishi samples. Interestingly, the size reduction was observed only in the spring of 2011 but not in the fall of 2011 or afterwards. Early exposure to high-energy short-lived radionuclides might have played a major role in this phenomenon.

A gap in the body size distribution was defined at the southernmost localities between which significant differences in body size were found in the Tohoku district (Taira et al. 2015). A body size distribution gap was found between Miyagi and Fukushima Prefectures in 2012 and 2013 (Figure 1). Northern populations beyond this gap have a smaller body size than southern populations. Shiroishi is likely in the transition zone, which is located in Miyagi Prefecture but close to the prefectural boundary. Fukushima belongs to the southern populations. However, as mentioned above, in the spring of 2011, the body size of the Fukushima samples was significantly smaller than that of the Shirosishi samples (Hiyama et al. 2012a, 2013), and this relationship was reversed in the fall of 2012; the Fukushima samples were larger than the Shiroishi samples in this year, although the difference was not statistically significant (Taira et al. 2015). Indeed, a size difference was not detected in the fall of 2011 or later (data not shown). Although the spring morph is generally larger than the summer (fall) morph in this butterfly, this seasonal effect did not play a role in the size difference between 2 localities in a given season. Therefore, we conclude that the small body size of the Fukushima samples detected in the spring of 2011 likely originated as a consequence of the nuclear accident.

Morphological Abnormalities as Genetic and Physiological Effects

Considering the sampling dates, experimental conditions, and nature of the correlations that were obtained, it is possible to infer the possible mechanisms manifesting the abnormalities (Taira et al. 2014). Abnormalities are consequences of genetic effects (inherited germ-line mutations of genes) and/or physiological effects (including general stress and somatic mutations of genes). For example, abnormalities found in the first-generation butterflies after the nuclear accident caught from the polluted areas are all caused by physiological damage (likely with somatic mutations) but not by genetic damage because these butterflies were irradiated at the larval and pupal stages to manifest adult abnormalities, during which no reproductive germ-line transmission was experienced. However, their germ-line cells might have been damaged, which is manifested as abnormalities in the laboratory-reared offspring generations from the field-caught females. Similarly, abnormalities that were manifested in the fifth-generation field-caught butterflies after the nuclear accident were likely caused by a mixture of genetic and physiological effects.

Any spontaneous abnormalities that were found throughout the Kanto-Tohoku districts could be due to both genetic and physiological effects. However, assuming that environmental stress is not very high in nonpolluted areas, such spontaneous background-level abnormalities may be caused by genetic deterioration (i.e., inbreeding depression) from repeated sibling crosses within a small locally isolated population (Hiyama et al. 2010; Iwata et al. 2013).

Temporal Dynamics and Adaptive Evolution

According to Hiyama et al. (2015), after the Fukushima nuclear accident, the abnormality rate peaked in the fall of 2011 and then decreased after that despite continuous exposure from radioactive cesium. Chronic cesium exposure is unlikely to cause extensive germ-line genetic damage (Nohara et al. 2014). Germ-line genetic mutations were primarily caused by high-energy short-lived radionuclides immediately after the accident. These mutations were inherited by the offspring generations. However, despite the acute and chronic exposures to environmental radiation, the butterfly populations did not go extinct, pointing out the possibility of adaptive evolution of the butterfly populations (Nohara et al. 2017).

Here, we present results of Hiyama et al. (2015) from a new point of view that was not presented in the original article. At first glance, a plot of the abnormality rate in response to the ground radiation dose (count rate) did not seem to suggest any relationship. However, when the data were divided into three stages based on temporal sequence, a clear picture emerges. The data points from spring 2011 (here defined as the immediately early stage) exhibited a high correlation between abnormality and the ground radiation dose (Spearman correlation coefficient $\rho = 0.95$; $P = 0.014$; $n = 5$) (Figure 2A), which likely indicates dose-dependent physiological effects including somatic mutations by high-energy short-lived radionuclides. However, the regression line for the immediate early stage exhibited a relatively small coefficient value for x (i.e., 0.0058), indicating that the dose effects were not very high. Likewise, the data points from fall 2011 and spring 2012 (here defined as the early stage) also exhibited a reasonable correlation (Spearman correlation coefficient $\rho = 0.67$; $P = 0.023$; $n = 11$) (Figure 2A). Interestingly, they were fit with a very different regression line with a larger coefficient value for x (i.e., 0.020), which may suggest an "amplification" of the dose effects (an approximately 4-fold increase) caused by the germ-line mutations from the

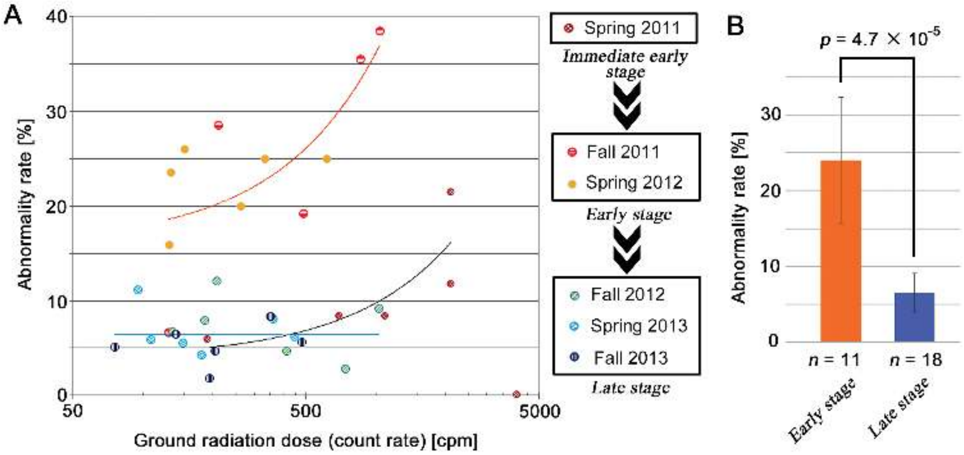

Figure 2. Relationship between the abnormality rate in the field-caught adult individuals (%) and ground radiation dose (count rate) (cpm) in 3 temporal stages (the immediately early stage, the early stage, and the late stage) over 3 years (2011–2013). Field sampling was performed twice a year (spring and fall). Statistical analyses were performed using R ver. 2.1.5.3 (The R Foundation for Statistical Computing, Vienna, Austria) except for regression lines and their associated R^2, which were obtained using Microsoft Excel. Normality of samples was checked with the Shapiro–Wilk test: shapiro.test(). Equality of variance was checked with the *F*-test: var.test(). (**A**) Scatter plot and simple regression lines for 3 stages. When one data point from the immediately early stage that has the largest ground radiation dose and zero abnormality rate (at Motomiya) was excluded as an outlier, the data points for the immediate early stage exhibit a reasonable fit with a regression line $y = 0.0058x + 3.9527$ ($R^2 = 0.65$) (shown as a black line) with a high Spearman correlation coefficient ($\rho = 0.95$; $P = 0.014$; $n = 5$). Data points for the early stage were reasonably fit with a regression line $y = 0.020x + 16.004$ ($R^2 = 0.53$) (shown as a red line) with a relatively high Spearman correlation coefficient ($\rho = 0.67$; $P = 0.023$; $n = 11$), whereas data points for the late stage were fit with a regression line $y = 0.00004x + 6.4719$ ($R^2 = 0.00002$) (shown as a blue line) with a low Spearman correlation coefficient ($\rho = -0.022$; $P = 0.93$; $n = 18$). Distribution ranges of the early stage (min. 130 to max. 1036) and late stages (min. 76 to max. 1031) were similar, although that of the immediate early stage was larger (min. 134 to max. 4050 and the second max. 2100). Spearman correlation coefficient was calculated using the following command: cor.test(X,Y, method = "spearman"). (**B**) Mean and standard deviation of the abnormality rate for the early stage (24.0 ± 8.4%) and the late stage (6.5 ± 2.6%). Difference was statistically significant ($t = 6.4$; df = 11; $P = 0.000047$). Welch's two-sample *t*-test was performed using the following command: t.test(X,Y, var.equal = F). See online version for full colors.

previous stage, leading to a deterioration of the populations in the early stage. In contrast, the subsequent data points from fall 2012, spring 2013, and spring 2013 (here defined as the late stage) did not show a significant correlation ($\rho = -0.022$; $P = 0.93$; $n = 18$), which suggests a shift from vulnerable to resistant stages. The difference in abnormality rates between these stages was statistically significant ($t = 6.4$, df = 11, $P = 0.000047$; Welch's two sample *t*-test) (Figure 2B). These results are explained most likely by the response changes of the butterfly from the immediate early stage to the early stage and then to the late stage. The latter change can be considered a process of adaptive evolution.

We believe that these discussions on the geographical and temporal dynamics of the pale grass blue butterflies or any other organisms are important to precisely understand the biological effects of the Fukushima nuclear accident. We thus stress the importance of monitoring indicator species from immediately after an accident to at least a few years afterwards, which was also discussed as one of the 3 lessons from Fukushima in Otaki (2016).

Towards the Whole Picture

Filling the Gap between Our Results and Conventional Understanding

In most dosimetric analyses, β-rays are mostly ignored, because they are easily shielded by a tissue surface layer of a few millimeters thickness, and thus γ-rays are considered most important to amount to effective dose. However, in the case of small animals such as insects including the pale grass blue butterfly, β-ray energy can be transferred to the whole body of the organisms, which could lead to serious adverse effects. This β-ray effect may be a reason that causes the high mortality and abnormality rates in the butterfly (Endo et al. 2014). A similar discussion can be found in Akimoto (2014) in explaining high abnormality rates in gall-forming aphids.

More important, an underlying assumption of many, if not most, radiation scientists and politicians, including ICRP and UNSCEAR, in discussing the possible effects of the Fukushima nuclear accident (and others) is that it is a direct radiation mechanism alone that causes any adverse biological effects. However, this assumption is unlikely to be correct in the real world. We believe that this is one of the important reasons for the "discrepancy" between our data and the conventional understanding of radiation biology, which is mostly based on laboratory experiments involving external irradiation.

To clarify the importance of the system differences, it is necessary to show that the pale grass blue butterfly is not an exceptionally weak animal when exposed to irradiation under the conventional experimental conditions. Insects in general have been considered highly resistant against irradiation at least under the conventional experimental conditions. If this butterfly is not affected much under the conventional experimental conditions, this means that the conventional radiological knowledge is applicable to this butterfly and that additional nondirect mechanisms are in effect in the wild to cause deaths and abnormalities even at low pollution levels. To demonstrate this point, a ^{60}Co γ-ray irradiation experiment may be conducted. Alternatively, artificial diets containing ^{137}Cs may be fed to larvae. On the other hand, we plan to examine whether the high sensitivity to Fukushima conditions that is observed in the pale grass blue butterfly in our feeding experiments can be observed in different organisms to demonstrate that this butterfly is not a peculiar organism in terms of radiation sensitivity.

Possible Unconventional Mechanisms

Then, what are the additional "indirect" mechanisms that cause high levels of deaths and abnormalities under field conditions? Laboratory environments and wild environments are certainly very different. In the wild, there are various stressors, for example, various predators, chemical pollutants, and climatic conditions (Mothersill et al. 2007; Manti and D'Arco 2010), as discussed in Garnier-Laplace et al. (2013). However, our feeding experiments were performed in the laboratory with no predators and no other known pollutants under constant "climate" conditions. Therefore, in this case, the causality of high mortality and abnormality rates in these experiments should be confined to the contaminated leaves.

As discussed in Otaki (2016), a nuclear accident may be considered a case of particulate matter air pollution. Radioactive nanoparticles were released from the Fukushima Daiichi Nuclear Power Plant (Adachi et al. 2013; Niimura et al. 2015; Abe et al. 2014; Yamaguchi et al. 2016), and they were distributed as an atmospheric aerosol throughout the world (Malá et al. 2013; Masson et al. 2013; Miyamoto et al. 2014; Muramatsu et al. 2015). The particles do not have to be radioactive to cause physiological disturbances in animals. The particles will be adsorbed onto the surface of leaves (Yamaguchi et al. 2012; Mimura et al. 2014) and may then be ingested or inhaled by butterfly larvae. When ingested or inhaled, these particles could cause innate immune responses, at least theoretically. When the particles behave as an epitope, adaptive immune response may also be triggered in humans (Seaton et al. 1995; Kappos et al. 2004; Utell and Frampton 2000; Shiraiwa et al. 2012) and in other vertebrates. Accompanying radioactive materials may make benign proteins antigenic through the radiation-induced denaturing of proteins to trigger adaptive immune responses.

A totally different mechanism, an indirect nutritional effect, may also exist. Thiamine (vitamin B_1) deficiency has been implicated as a mechanism of impacts of chemical pollution in birds and other wild animals (de Roode et al. 2000; Balk et al. 2009; Sonne et al. 2012; Turja et al. 2014). Similarly, it is possible that the leaves of the host plant of the butterfly, *O. corniculata*, do not synthesize enough thiamine for larvae after exposure to long-term low-dose irradiation despite no notable morphological abnormalities being manifested in the plant. Larvae that eat the thiamine-deficient leaves could die because of lack of thiamine.

Other unknown pathways may exist. Synergistic effects are also possible. We propose that there could be several different unknown pathways that could harm biota. For example, unknown symbiotic relationships with intestinal bacteria may be affected directly or indirectly. At this point, we do not know the whole picture of the pollution effects. Therefore, we have to study Fukushima from multiple aspects, including but not limited to the conventional radiation effect, to obtain the whole picture of the biological impacts of the Fukushima nuclear accident, which was stressed as one of the 3 lessons from Fukushima in Otaki (2016). Our results regarding the butterfly would be more or less applicable to other organisms, including humans.

Funding

We greatly thank act beyond trust (Tokyo) and the Asahi Glass Foundation (Tokyo) for providing financial assistance for field work and laboratory experiments after the Fukushima nuclear accident. We also greatly thank general public funders who philanthropically supported our studies.

Acknowledgments

We are grateful to the members of the BCPH Unit of Molecular Physiology, especially A Hiyama, K Sakauchi, C Nohara, M Iwasaki, and M Iwata, for their participation in the field work and A Tanahara for technical advice. We also greatly thank K Yoshida, N Itoh, Y Nagahata, and other volunteers and donators who appreciate the importance of our research after the accident. We also thank TY Steen for the invitation of this article.

References

Abe Y, Iizawa Y, Terada Y, Adachi K, Igarashi Y, Nakai I. 2014. Detection of uranium and chemical state analysis of individual radioactive microparticles emitted from the Fukushima nuclear accident using multiple synchrotron radiation X-ray analyses. *Anal Chem*. 86:8521–8525.

Adachi K, Kajino M, Zaizen Y, Igarashi Y. 2013. Emission of spherical cesium-bearing particles from an early stage of the Fukushima nuclear accident. *Sci Rep*. 3:2554.

Ahola V, Lehtonen R, Somervuo P, Salmela L, Koskinen P, Rastas P, Välimäki N, Paulin L, Kvist J, Wahlberg N, *et al*. 2014. The Glanville fritillary genome retains an ancient karyotype and reveals selective chromosomal fusions in Lepidoptera. *Nat Commun*. 5:4737.

Akimoto S. 2014. Morphological abnormalities in gall-forming aphids in a radiation-contaminated area near Fukushima Daiichi: selective impact of fallout? *Ecol Evol*. 4:355–369.

Balk L, Hägerroth PA, Akerman G, Hanson M, Tjärnlund U, Hansson T, Hallgrimsson GT, Zebühr Y, Broman D, Mörner T, *et al*. 2009. Wild birds of declining European species are dying from a thiamine deficiency syndrome. *Proc Natl Acad Sci U S A*. 106:12001–12006.

Bonisoli-Alquati A, Koyama K, Tedeschi DJ, Kitamura W, Sukuzi H, Ostermiller S, Arai E, Møller AP, Mousseau TA. 2015. Abundance and genetic damage of barn swallows from Fukushima. *Sci Rep*. 5:9432.

Buckley J, Bridle JR, Pomiankowski A. 2010. Novel variation associated with species range expansion. *BMC Evol Biol*. 10:382.

Callaway E. 2013. Fukushima offers real-time ecolab. *Nature*. 499:265–266.

Cong Q, Borek D, Otwinowski Z, Grishin NV. 2015a. Tiger swallowtail genome reveals mechanisms for speciation and caterpillar chemical defense. *Cell Rep*. 10:910–919.

Cong Q, Borek D, Otwinowski Z, Grishin NV. 2015b. Skipper genome sheds light on unique phenotypic traits and phylogeny. *BMC Genomics*. 16:639.

Cong Q, Shen J, Borek D, Robbins RK, Otwinowski Z, Grishin NV. 2016a. Complete genomes of Hairstreak butterflies, their speciation, and nucleo-mitochondrial incongruence. *Sci Rep*. 6:24863.

Cong Q, Shen J, Warren AD, Borek D, Otwinowski Z, Grishin NV. 2016b. Speciation in cloudless sulphurs gleaned from complete genomes. *Genome Biol Evol*. 8:915–931.

de Roode DF, Balk L, Koeman JH. 2000. Development of a bioassay to test the possible role of thiamine disturbance as a mechanism behind pollution-induced reproductive failures in birds. *Arch Environ Contam Toxicol*. 39:386–391.

Eisenhardt D. 2014. Molecular mechanisms underlying formation of long-term reward memories and extinction memories in the honeybee (*Apis mellifera*). *Learn Mem*. 21:534–542.

Elsik CG, Worley KC, Bennett AK, Beye M, Camara F, Childers CP, de Graaf DC, Debyser G, Deng J, Devreese B, *et al*.; Honey Bee Genome Sequencing Consortium. 2014. Finding the missing honey bee genes: lessons learned from a genome upgrade. *BMC Genomics*. 15:86.

Endo S, Tanaka K, Kajimoto T, Thanh NT, Otaki JM, Imanaka T. 2014. Estimation of β-ray dose in air and soil from Fukushima Daiichi Power Plant accident. *J Radiat Res*. 55:476–483.

Fukunaga H, Yokoya A. 2016. Low-dose radiation risk and individual variation in radiation sensitivity in Fukushima. *J Radiat Res*. 57:98–100.

Garnier-Laplace J, Beaugelin-Seiller K, Della-Vedova C, Métivier JM, Ritz C, Mousseau TA, Møller AP. 2015. Radiological dose reconstruction for birds reconciles outcomes of Fukushima with knowledge of dose–effect relationships. *Sci Rep*. 5:16594.

Garnier-Laplace J, Geras'kin S, Della-Vedova C, Beaugelin-Seiller K, Hinton TG, Real A, Oudalova A. 2013. Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *J Environ Radioact*. 121:12–21.

Hayama S, Nakiri S, Nakanishi S, Ishii N, Uno T, Kato T, Konno F, Kawamoto Y, Tsuchida S, Ochiai K, *et al*. 2013. Concentration of radiocesium in the wild Japanese monkey (*Macaca fuscata*) over the first 15 months after the Fukushima Daiichi nuclear disaster. *PLoS One*. 8:e68530.

Hayashi G, Shibato J, Imanaka T, Cho K, Kubo A, Kikuchi S, Satoh K, Kimura S, Ozawa S, Fukutani S, *et al*. 2014. Unraveling low-level gamma radiation–responsive changes in expression of early and late genes in leaves of rice seedlings at Iitate Village, Fukushima. *J Hered*. 105:723–738.

Heliconius Genome Consortium. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*. 487:94–98.

Hiyama A, Iwata M, Otaki JM. 2010. Rearing the pale grass blue *Zizeeria maha* (Lepidoptera, Lycaenidae): Toward the establishment of a lycaenid model system for butterfly physiology and genetics. *Entomol Sci*. 13:293–302.

Hiyama A, Nohara C, Kinjo S, Taira W, Gima S, Tanahara A, Otaki JM. 2012a. The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. *Sci Rep*. 2:570.

Hiyama A, Nohara C, Taira W, Kinjo S, Iwata M, Otaki JM. 2013. The Fukushima nuclear accident and the pale grass blue butterfly: evaluating biological effects of long-term low-dose exposures. *BMC Evol Biol*. 13:168.

Hiyama A, Taira W, Otaki JM. 2012b. Color-pattern evolution in response to environmental stress in butterflies. *Front Genet*. 3:15.

Hiyama A, Taira W, Iwasaki M, Sakauchi K, Gurung R, Otaki JM. 2017. Geographical distribution of morphological abnormalities and wing color pattern modifications of the pale grass blue butterfly in northeastern Japan. *Entomol Sci*. 20:100–110.

Hiyama A, Taira W, Nohara C, Iwasaki M, Kinjo S, Iwata M, Otaki JM. 2015. Spatiotemporal abnormality dynamics of the pale grass blue butterfly: three years of monitoring (2011–2013) after the Fukushima nuclear accident. *BMC Evol Biol*. 15:15.

Honey Bee Genome Sequencing Consortium. 2006. Insights into social insects from the genome of the honey bee *Apis mellifera*. *Nature*. 443:931–949.

Horiguchi T, Yoshii H, Mizuno S, Shiraishi H. 2016. Decline in intertidal biota after the 2011 Great East Japan Earthquake and Tsunami and the Fukushima nuclear disaster: field observations. *Sci Rep*. 6:20416.

International Commission on Radiological Protection. 2007. Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. *Ann ICRP*. 37:1–332.

International Commission on Radiological Protection. 2008. Environmental Protection—the concept and use of reference animals and plants. ICRP Publication 108. *Ann ICRP*. 38:3–242.

Inomata T, Uémura Y, Yago M, Jinbo U, Ueda K. 2010. The current checklist of Japanese butterflies. Available from: http://binran.lepimages.jp/species/324. Accessed October 2016.

Iwata M, Hiyama A, Otaki JM. 2013. System-dependent regulations of colour-pattern development: a mutagenesis study of the pale grass blue butterfly. *Sci Rep*. 3:2379.

Kappos AD, Bruckmann P, Eikmann T, Englert N, Heinrich U, Höppe P, Koch E, Krause GH, Kreyling WG, Rauchfuss K, *et al*. 2004. Health effects of particles in ambient air. *Int J Hyg Environ Health*. 207:399–407.

Kunte K, Zhang W, Tenger-Trolander A, Palmer DH, Martin A, Reed RD, Mullen SP, Kronforst MR. 2014. *doublesex* is a mimicry supergene. *Nature*. 507:229–232.

Lelieveld J, Kunkel D, Lawrence MG. 2012. Global risk of radioactive fallout after major nuclear reactor accidents. *Atoms Chem Phys*. 12:4245–4258.

Li X, Fan D, Zhang W, Liu G, Zhang L, Zhao L, Fang X, Chen L, Dong Y, Chen Y, *et al*. 2015. Outbred genome sequencing and CRISPR/Cas9 gene editing in butterflies. *Nat Commun*. 6:8212.

Malá H, Rulík P, Bečková V, Mihalík J, Slezáková M. 2013. Particle size distribution of radioactive aerosols after the Fukushima and the Chernobyl accidents. *J Environ Radioact*. 126:92–98.

Manti L, D'Arco A. 2010. Cooperative biological effects between ionizing radiation and other physical and chemical agents. *Mutat Res*. 704:115–122.

Masson O, Ringer W, Malá H, Rulik P, Dlugosz-Lisiecka M, Eleftheriadis K, Meisenberg O, De Vismes-Ott A, Gensdarmes F. 2013. Size distributions of airborne radionuclides from the Fukushima nuclear accident at several places in Europe. *Environ Sci Technol*. 47:10995–11003.

Mimura T, Mimura M, Kobayashi D, Komiyama C, Sekimoto H, Miyamoto M, Kitamura A. 2014. Radioactive pollution and accumulation of radionuclides in wild plants in Fukushima. *J Plant Res*. 127:5–10.

Miyamoto Y, Yasuda K, Magara M. 2014. Size distribution of radioactive particles collected at Tokai, Japan 6 days after the nuclear accident. *J Environ Radioact*. 132:1–7.

Møller AP, Mousseau TA. 2006. Biological consequences of Chernobyl: 20 years on. *Trends Ecol Evol*. 21:200–207.

Møller AP, Hagiwara A, Matsui S, Kasahara S, Kawatsu K, Nishiumi I, Suzuki H, Ueda K, Mousseau TA. 2012. Abundance of birds in Fukushima as judged from Chernobyl. *Environ Pollut*. 164:36–39.

Møller AP, Mousseau TA. 2013a. The effects of natural variation in background radioactivity on humans, animals and other organisms. *Biol Rev Camb Philos Soc*. 88:226–254.

Møller AP, Mousseau TA. 2013b. Low-dose radiation, scientific scrutiny, and requirements for demonstrating effects. *BMC Biol*. 11:92.

Møller AP, Nishiumi I, Suzuki H, Ueda K, Mousseau T. 2013. Differences in effects of radiation on abundance of animals in Fukushima and Chernobyl. *Ecol Indic*. 24:75–81.

Mothersill C, Salbu B, Heier LS, Teien HC, Denbeigh J, Oughton D, Rosseland BO, Seymour CB. 2007. Multiple stressor effects of radiation and metals in salmon (Salmo salar). *J Environ Radioact*. 96:20–31.

Mousseau TA, Møller AP. 2014. Genetic and ecological studies of animals in Chernobyl and Fukushima. *J Hered*. 105:704–709.

Muramatsu H, Kawasumi K, Kondo T, Matsuo K, Itoh S. 2015. Size-distribution of airborne radioactive particles from the Fukushima accident. *J Radioanal Nucl Chem*. 303:1459–1463.

Murase K, Murase J, Horie R, Endo K. 2015. Effects of the Fukushima Daiichi nuclear accident on goshawk reproduction. *Sci Rep*. 5:9405.

Niimura N, Kikuchi K, Tuyen ND, Komatsuzaki M, Motohashi Y. 2015. Physical properties, structure, and shape of radioactive Cs from the Fukushima Daiichi Nuclear Power Plant accident derived from soil, bamboo and shiitake mushroom measurements. *J Environ Radioact*. 139:234–239.

Nishikawa H, Iijima T, Kajitani R, Yamaguchi J, Ando T, Suzuki Y, Sugano S, Fujiyama A, Kosugi S, Hirakawa H, *et al*. 2015. A genetic mechanism for female-limited Batesian mimicry in Papilio butterfly. *Nat Genet*. 47:405–409.

Nohara C, Hiyama A, Taira W, Otaki JM. 2018. Robustness and radiation resistance of the pale grass blue butterfly from radioactively contaminated areas: a possible case of adaptive evolution. *J Hered*. 109:188–198.

Nohara C, Hiyama A, Taira W, Tanahara A, Otaki JM. 2014a. The biological impacts of ingested radioactive materials on the pale grass blue butterfly. *Sci Rep*. 4:4946.

Nohara C, Taira W, Hiyama A, Tanahara A, Takatsuji T, Otaki JM. 2014b. Ingestion of radioactively contaminated diets for two generations in the pale grass blue butterfly. *BMC Evol Biol*. 14:193.

Ochiai K, Hayama S, Nakiri S, Nakanishi S, Ishii N, Uno T, Kato T, Konno F, Kawamoto Y, Tsuchida S, *et al*. 2014. Low blood cell counts in wild Japanese monkeys after the Fukushima Daiichi nuclear disaster. *Sci Rep*. 4:5793.

Otaki JM. 2016. Fukushima's lessons from the blue butterfly: a risk assessment of the human living environment in the post-Fukushima era. *Integr Environ Assess Manag*. 12:667–672.

Otaki JM, Hiyama A, Iwata M, Kudo T. 2010. Phenotypic plasticity in the range-margin population of the lycaenid butterfly *Zizeeria maha*. *BMC Evol Biol*. 10:252.

Parmesan C. 2003. Butterflies as bioindicators for climate change effects. In: Boggs CL, Watt WB, Ehrlich PR, editors. *Butterflies: ecology and evolution taking flight*. Chicago (IL): The University of Chicago Press. p. 541–560.

Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, Huntley B, Kaila L, Kullberg J, Tammaru T, *et al*. 1999. Polewards shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. 399:579–583.

Sánchez-Bayo F, Goulson D, Pennacchio F, Nazzi F, Goka K, Desneux N. 2016. Are bee diseases linked to pesticides? A brief review. *Environ Int*. 89–90:7–11.

Seaton A, MacNee W, Donaldson K, Godden D. 1995. Particulate air pollution and acute health effects. *Lancet*. 345:176–178.

Shiraiwa M, Selzle K, Pöschl U. 2012. Hazardous components and health effects of atmospheric aerosol particles: reactive oxygen species, soot, polycyclic aromatic compounds and allergenic proteins. *Free Radic Res*. 46:927–939.

Shirôzu T. 2006. *The standard of butterflies in Japan*. Tokyo (Japan): Gakken (In Japanese).

Sonne C, Alstrup AK, Therkildsen OR. 2012. A review of the factors causing paralysis in wild birds: implications for the paralytic syndrome observed in the Baltic Sea. *Sci Total Environ*. 416:32–39.

Taira W, Hiyama A, Nohara C, Sakauchi K, Otaki JM. 2015a. Ingestional and transgenerational effects of the Fukushima nuclear accident on the pale grass blue butterfly. *J Radiat Res*. 56(Suppl 1):i2–18.

Taira W, Iwasaki M, Otaki JM. 2015b. Body size distributions of the pale grass blue butterfly in Japan: size rules and the status of the Fukushima population. *Sci Rep*. 5:12351.

Taira W, Nohara C, Hiyama A, Otaki JM. 2014. Fukushima's biological impacts: the case of the pale grass blue butterfly. *J Hered*. 105:710–722.

Turja R, Höher N, Snoeijs P, Baršienė J, Butrimavičienė L, Kuznetsova T, Kholodkevich SV, Devier MH, Budzinski H, Lehtonen KK. 2014. A multi-biomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (*Mytilus trossulus*). *Sci Total Environ*. 473–474:398–409.

United Nations Scientific Committee on the Effects of Atomic Radiation. 2008. *Sources and effects of ionizing radiation. Volume II: effects. Scientific Annexes C, D and E. UNSCEAR 2008 report*. New York (NY): United Nations.

United Nations Scientific Committee on the Effects of Atomic Radiation. 2013. *Sources, and effects and risks of ionizing radiation. Volume I: report to the general assembly, Scientific Annex A. UNSCEAR 2013 report*. New York (NY): United Nations.

United Nations Scientific Committee on the Effects of Atomic Radiation. 2015. *Developments since the 2013 UNSCEAR Report on the levels and effects of radiation exposure due to the nuclear accident following the Great East-Japan Earthquake and Tsunami. A 2015 white paper to guide the Scientific Committee's future programme of work*. New York (NY): United Nations.

United Nations Scientific Committee on the Effects of Atomic Radiation. 2016. *Developments since the 2013 UNSCEAR Report on the levels and effects of radiation exposure due to the nuclear accident following the Great East-Japan Earthquake and Tsunami. A 2016 white paper to guide the Scientific Committee's future programme of work*. New York (NY): United Nations.

Utell MJ, Frampton MW. 2000. Acute health effects of ambient air pollution: the ultrafine particle hypothesis. *J Aerosol Med*. 13:355–359.

Watanabe Y, Ichikawa S, Kubota M, Hoshino J, Kubota Y, Maruyama K, Fuma S, Kawaguchi I, Yoschenko VI, Yoshida S. 2015. Morphological defects in native Japanese fir trees around the Fukushima Daiichi Nuclear Power Plant. *Sci Rep*. 5:13232.

Wheatley S, Sovacool B, Sornette D. 2017. Of disasters and dragon kings: a statistical analysis of nuclear power incidents and accidents. *Risk Anal*. 37:99–115.

Yago K, Odagiri K. 2007. Lycaenidae. In: Yata O, editor. *Iconographia Insectorum Japonicorum Colore Naturali Edita*. Vol. I. Tokyo (Japan): Hokuryukan. p. 148–153. (in Japanese).

Yamaguchi N, Eguchi S, Fujiwara H, Hayashi K, Tsukada H. 2012. Radiocesium and radioiodine in soil particles agitated by agricultural practices: field observation after the Fukushima nuclear accident. *Sci Total Environ*. 425:128–134.

Yamaguchi N, Mitome M, Kotone AH, Asano M, Adachi K, Kogure T. 2016. Internal structure of cesium-bearing radioactive microparticles released from Fukushima nuclear power plant. *Sci Rep*. 6:20548.

Zhan S, Merlin C, Boore JL, Reppert SM. 2011. The monarch butterfly genome yields insights into long-distance migration. *Cell*. 147:1171–1185.