



Original Article

Robustness and Radiation Resistance of the Pale Grass Blue Butterfly from Radioactively Contaminated Areas: A Possible Case of Adaptive Evolution

Chiyo Nohara, Atsuki Hiyama, Wataru Taira, and Joji M. Otaki

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

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

Received September 6, 2016; First decision December 6, 2016; Accepted February 7, 2017.

Corresponding Editor: Tomoko Steen

Abstract

The pale grass blue butterfly, *Zizeeria maha*, has been used to evaluate biological impacts of the Fukushima nuclear accident in March 2011. Here, we examined the possibility that butterflies have adapted to be robust in the contaminated environment. Larvae ($n = 2432$) were obtained from adult butterflies ($n = 20$) collected from 7 localities with various contamination levels in May 2012, corresponding to the 7th generation after the accident. When the larvae were reared on non-contaminated host plant leaves from Okinawa, the normality rates of natural exposure without artificial irradiation (as an indication of robustness) were high not only in the least contaminated locality but also in the most contaminated localities. The normality rates were similarly obtained when the larvae were reared on non-contaminated leaves with external irradiation or on contaminated leaves from Fukushima to deliver internal irradiation. The normality rate of natural exposure and that of external or internal exposure were correlated, suggesting that radiation resistance (or susceptibility) likely reflects general state of health. The normality rate of external or internal exposure was divided by the relative normality rate of natural exposure, being defined as the resistance value. The resistance value was the highest in the populations of heavily contaminated localities and was inversely correlated with the distance from the Fukushima Dai-ichi nuclear power plant. These results suggest that the butterfly population might have adapted to the contaminated environment within approximately 1 year after the accident. The present study may partly explain the decrease in mortality and abnormality rates later observed in the contaminated areas.

Subject area: Conservation genetics and biodiversity, Molecular adaptation and selection

Key words: Fukushima nuclear accident, pale grass blue butterfly, radiation resistance, radioactive pollution, robustness, *Zizeeria maha*

The Fukushima nuclear accident in March 2011 caused widespread nuclear pollution (Chino et al. 2011; Kinoshita et al. 2011; Hirose 2012; Torii et al. 2013). Particularly polluted areas are the

Fukushima and Ibaraki Prefectures in the southern Tohoku district and the northern Kanto district near the Fukushima Dai-ichi nuclear power plant (NPP). Several field studies have already demonstrated

the effects of the Fukushima pollution on various organisms; the bird and arthropod populations (Møller et al. 2012, 2013), the intertidal species populations including the rock shell (Horiguchi et al. 2016), lycaenid butterfly (Hiyama et al. 2012a, 2013, 2015; Nohara et al. 2014a, 2014b; Taira et al. 2014, 2015a; Otaki 2016), gall-forming aphids (Akimoto 2014), Japanese monkey (Ochiai et al. 2014), barn swallow (Bonisoli-Alquati et al. 2015), goshawk (Murase et al. 2015), rice plant (Hayashi et al. 2014), and fir tree (Watanabe et al. 2015).

Radioactive materials, whether artificial or natural, are generally considered mutagenic for organisms because of their ability to cause mutations via ionizing radiation (Muller 1927, 1928; Møller and Mousseau 2013a, 2013b). This effect is particularly relevant for organisms that live in the heavily polluted areas. Mutagens that suddenly emerge in an environment will likely cause random mutations in somatic and germ-line cells; in the former case, chronic diseases such as thyroid cancer may emerge, which was demonstrated by the Chernobyl nuclear accident (Pacini et al. 1997); in the latter case, offspring generations may be genetically affected, as was found in swallows in Chernobyl (Ellegren et al. 1997). Allelic diversity produced by such germ-line mutations may initiate selection for adaptive evolution according to a modern view of evolutionary biology (Barton et al. 2007; Hillis et al. 2012; Futuyma 2013; Zimmer and Emlen 2013). In addition, maternal effects from various environmental factors, possibly including radiation levels, are known to play a critical role in the offspring fitness, which is subjected to natural selection (Mousseau and Dingle 1991; Mousseau and Fox 1998). These evolutionary aspects of biological dynamics have not been adequately stressed in evaluating the impacts of nuclear pollution (Galván et al. 2014), but these aspects are important to precisely understand what has occurred and to predict what will occur in organisms that live in the polluted areas. The nuclear accident provides evolutionary biologists with real-world (i.e., non-theoretical) and real-time opportunities to examine the evolutionary process in the field (Callaway 2013).

In the history of biology, butterflies and moths have often contributed to the development of evolutionary concepts. For example, Brazilian butterflies collected by Bates and Müller inspired them to discover Batesian and Müllerian mimicry, respectively, which contributed to establishing the Darwinian theory of natural selection (Wickler 1968). The genetic, developmental and ecological studies of the *Heliconius* butterflies, a genus displaying Müllerian mimicry, have accumulated molecular evidence for natural selection (Heliconius Genome Consortium 2012; Nadeau et al. 2016). The most famous field observation of the natural selection process is perhaps a case of industrial melanization of the peppered moth (Majerus 1998; Cook 2003; van't Hof et al. 2011, 2016; Cook et al. 2012; Cook and Saccheri 2013). Currently, butterflies and moths are used for multiple disciplines, such as developmental biology, evolutionary biology, ecology, behavioral biology, and environmental sciences (Nijhout 1991; Ehrlich 2003). In contrast to nymphalid butterflies, which have often been the focus of these studies, we have established a lycaenid model butterfly that can be useful for physiological, genetic, and environmental studies, at least in Japan: the pale grass blue butterfly, *Zizeeria maha* (Hiyama et al. 2010). This butterfly has also been used for ecological risk assessment of transgenic plants (Shirai and Takahashi 2005; Wolt et al. 2015).

The pale grass blue butterfly is widely distributed in Japan, except in Hokkaido (Shirôzu 2006; Yata 2007), and coexists with humans; the butterfly can be found even in a metropolitan area such as Tokyo (Washitani et al. 2013), which makes it a useful indicator

for human-living environments. The natural history of this butterfly is well known (Oda and Kitazoe 2002). It is multivoltine, completing 1 generation in 1 month. Importantly, this butterfly is amenable for large-scale field collection and laboratory experiments. It was used to study range expansion, phenotypic plasticity and evolution (Otaki et al. 2010; Buckley et al. 2010; Hiyama et al. 2012b) and the developmental effects of mutagenesis (Iwata et al. 2013).

Furthermore, the pale grass blue butterfly has been used to examine the biological effects of radioactive pollution from the Fukushima nuclear accident (Hiyama et al. 2012a, 2013, 2015, 2017; Nohara et al. 2014a, 2014b; Taira et al. 2014, 2015a; Otaki 2016). A series of field surveys, laboratory experiments, and discussions from multidisciplinary perspectives established the causality of the Fukushima Dai-ichi nuclear accident to high mortality and morphological abnormality of the pale grass blue butterfly that was detected particularly in the fall of 2011 (Hiyama et al. 2012a, 2013, 2015; Nohara et al. 2014a, 2014b; Taira et al. 2014). Importantly, when butterfly larvae from a least contaminated area (i.e., Okinawa) were irradiated externally by an artificial radiation source of ^{137}Cs or internally by feeding them host plant leaves from highly contaminated areas (i.e., Iitate, Fukushima, and Hirono), the survival rate decreased dose-dependently (Hiyama et al. 2012a). Moreover, adult butterflies that survived through the irradiation treatments showed phenotypic abnormalities that were also found in field-caught adults from highly contaminated areas (Hiyama et al. 2012a). According to the Postulates of Pollutant-Induced Biological Impact (Taira et al. 2014), which were proposed in reference to Koch's postulates of infectious disease, the following 6 criteria must be satisfied to prove that an environmental pollutant (or a group of pollutants) in question is a causal factor: 1) spatial relationship, 2) temporal relationship, 3) direct exposure, 4) phenotypic variability or spectrum, 5) experimental reproduction (external exposure), and 6) experimental reproduction (internal exposure). These 6 criteria have been well satisfied in the pale grass blue butterfly. Additionally, the transgenerational inheritance of abnormal traits has been shown in this butterfly; the Fukushima progeny (the F_2 generation) fed non-contaminated leaves exhibited inheritance of morphological abnormalities with low survival rate (Hiyama et al. 2012a; Nohara et al. 2014b).

Interestingly, the mortality and morphological abnormality of this butterfly sharply increased by the fall of 2011 but subsequently decreased to the normal level in the polluted localities by 2013 (Hiyama et al. 2015). A gradual decrease in radioactive materials by radioactive decay and physical dispersion may have partly contributed to these mortality and abnormality dynamics, but this explanation is not satisfactory, because radioactive materials that are sufficient to induce mortality and abnormality remain in the polluted areas (Hiyama et al. 2015). Moreover, there was a time delay in the abnormality peak of the butterfly from the dose peak (Hiyama et al. 2015), suggesting a non-acute mode of effects. A reasonable hypothesis is that the natural selection for radioactive resistance resulted in the adaptive evolution of the pale grass blue butterfly. If so, it is important to test whether the pale grass blue butterfly from the polluted areas is robust and whether it is resistant to radiation stress.

In this study, we collected adult butterflies from 7 localities (Tsukuba, Mito, Takahagi, Iwaki, Hirono, Motomiya, and Fukushima) with various contamination levels in May 2012 (14 months after the accident) and examined how well their offspring larvae can survive and become normal adults when eating non-contaminated host plant leaves. The offspring performance was further tested by feeding the larvae non-contaminated leaves together with

exposure to external radiation (external exposure experiment) and by feeding the larvae contaminated leaves from Fukushima (internal exposure experiment). We then examined the possible correlation of resistance with the ground radiation dose or with the distance from the Fukushima Dai-ichi NPP. We believe that such studies that probe dynamic changes of local populations at a reasonable time-point after a nuclear accident are scarce but very important to understand biological consequences of a nuclear accident in the field.

Materials and Methods

Ethics Statement

No specific permission is required to study the pale grass blue butterfly in Japan.

Butterflies

The pale grass blue butterfly *Z. maha* (Kollar 1844) (Lepidoptera, Lycaenidae) (Oda and Kitazoe 2002; Shirôzu 2006; Yata 2007) was used throughout this study. This monophagous butterfly thrives on the host plant *Oxalis corniculata*. This butterfly is multivoltine and completes 1 generation in a month. Except for in the winter season, this butterfly species completes its life cycle approximately 7 times a year in the southern Tohoku and northern Kanto districts. Natural history of this butterfly in Japan has been described briefly in Hiyama et al. (2013, 2015) and Taira et al. (2014, 2015).

Field Work

We collected adult butterflies on 12–16 May 2012 (“Spring 2012”). The *Z. maha* populations of Spring 2012 correspond to the 7th generation after the Fukushima Dai-ichi NPP accident. The collection localities were Tsukuba City, Mito City, Takahagi City (these 3 localities belong to Ibaraki Prefecture), Iwaki City, Hirono Town, Motomiya City, and Fukushima City (these 4 localities belong to Fukushima Prefecture). The precise collection sites are shown in Hiyama et al. (2015). The field work and subsequent egg collection overlapped with Hiyama et al. (2012a). Abnormality rates of these collected samples and their offspring generations have already been reported in Hiyama et al. (2012a, 2015).

We collected host plant leaves on 30 May and 10 June 2012 from Watari (located 57.6–58.1 km away from the Fukushima Dai-ichi NPP), Fukushima City. Immediately after collection, they were stored in humidified bags and sent to the laboratory under refrigerated conditions. Upon arrival, they were immediately stored in a refrigerator until use. Non-contaminated Okinawa leaves were also collected from the University of the Ryukyus and its vicinity on similar dates. They were similarly stored in a different refrigerator. Ground radiation doses at the collection sites were measured at the 0-cm level using an ALOKA Scintillation survey meter TCS-161 (Hitachi Aloka Medical). The nearest host plant from the place where an adult butterfly was collected was identified, and the site of the plant was used as the dose measurement site.

Egg Collection and Larval Rearing

We followed the rearing method described in Hiyama et al. (2010) with some modifications (Hiyama et al. 2012a). The eggs were collected in a 30-cm cubic glass case from female adults. The number of females used for egg collection were: 4 for Fukushima, 2 for Motomiya, 2 for Hirono, 3 for Iwaki, 3 for Takahagi, 3 for Mito, and 3 for Tsukuba. Thus, total sample size of adult butterflies

was 20. Eggs were collected from each locality group of females using the host plant from Nishihara Town, Okinawa Prefecture. All females used for the egg collection had normal phenotype with no noticeable abnormality. We tried to collect as many eggs as possible in a given time. The number of eggs (and hence the number of larvae) that were obtained varied among the locality groups, but we used as many individuals as possible. Total sample size of larvae was 2432. The collected offspring population was divided into 3 groups: control group (no exposure) ($n = 1137$), external exposure group ($n = 601$), and internal exposure group ($n = 694$). Uneven sample numbers among these 3 groups were partly due to unpredictability of the total number of larvae to be obtained and our emphasis on the control group at the time of the experimentation. The genetic difference among these groups is negligible, because they were sorted randomly from the same parents. This sorting was performed at the 14th day after egg deposition, when the larvae were fully visible to the naked eye. Larvae were fed the host plant leaves collected from Nishihara Town, Okinawa Prefecture, except for the internal exposure experiment.

Morphological Examination

The adults that successfully eclosed were examined under a stereomicroscope for the normality or abnormality of morphological structures of the entire external body parts, including wing shapes, wing color patterns, appendages, and other visible structures. A normal adult individual was defined as one that did not have any detected abnormalities.

Normality Rate, Robustness, and Resistance

The number of morphologically normal adults, with respect to the number of starting larvae, was used to obtain the normality rate under the natural exposure conditions (no artificial irradiation). We also calculated the physiological robustness values (or simply “robustness” in this paper), where the normality rate of the Tsukuba population was simply set at 100%. This is because the Tsukuba population was the least polluted locality population among the local populations surveyed (and hence ideally it should score 100%) and because it indeed had the highest level of the normality rate (see below). The normality rates of the collection in May 2011 (“Spring 2011”) and September/October (“Fall 2011”) were obtained from Hiyama et al. (2012a).

We defined the value of resistance to external irradiation (R_{ex}); to obtain R_{ex} , the normality rate of the external exposure experiment was divided by the robustness. Similarly, we defined the value of resistance to the internal irradiation (R_{in}); to obtain R_{in} , the normality rate that was obtained in the internal exposure experiment was divided by the robustness. Use of the robustness (instead of the normality rate) in denominator was based on the assumption that irradiation effects on animals at very small levels only with natural irradiation (no artificial irradiation) may fluctuate due to subtleness of the effects.

External Exposure Experiment

We used ^{137}Cs (14.3 MBq) as a radiation source. Irradiation was performed for 24 days. The larval containers were placed so that any locality groups were equally subjected to irradiation. The exposed doses varied among individuals, depending on the place where the containers were placed in relation to the radiation source. The farthest container from the radiation source was measured at 20.4 $\mu\text{Sv/h}$, and its cumulative dose was 7.848 mSv for the irradiation

period of 384.71 h, which excluded times for container cleaning and replacement of host plant leaves. The above data were obtained using an ALOKA scintillation survey meter TCS-161 (Hitachi Aloka Medical). The closest container to the radiation source was measured using DoseRAE2 PRM-1200 (RAE Systems, United States of America), which yielded 323.0 $\mu\text{Sv/h}$. The cumulative dose was 124.26 mSv for the irradiation period of 384.71 h. Thus, the range of irradiation was 7.85 mSv (minimum) to 124.26 mSv (maximum). Despite this large range, all locality groups were equally irradiated, which made direct comparisons among the locality groups possible. Irradiation was continuously executed in the prepupal and pupal stages until immediately before eclosion. Because of a small number of usable larvae from the Mito females, the external exposure experiment was performed using the 6 localities, excluding the Mito population.

Internal Exposure Experiment

The larvae were fed contaminated leaves from a single source, Watari, Fukushima City, from the 14th day after the egg deposition, immediately after the random sorting process, until eclosion. The amounts of radioactive cesium species (^{134}Cs and ^{137}Cs) of the host plant leaves from Watari, Fukushima City, were measured using a Canberra germanium semiconductor detector GCW-4023. A portion of the leaf samples given to larvae was saved every time, and these leaves were collectively dried, burned into ashes without flame, and subjected to measurements as described (Hiyama et al. 2012a).

This internal exposure experiment through ingestion of contaminated leaves was performed for all 7 localities.

Statistical Analysis

Scatter plots and other graphic representations were made using Microsoft Excel. Because of possible nonlinearity of radiation effects in animals, Spearman correlation coefficient ρ was obtained using the R version 3.1.3 (Austrian Association for Statistical Computing, Vienna, Austria).

Results

Distances and Ground Radiation Levels of the Localities Surveyed

We collected adult butterflies from the 7 localities in the southern Tohoku and northern Kanto districts in May 2012 (Figure 1A, B), which corresponded to the 7th generation after the Fukushima nuclear accident. Their distances from the Fukushima Dai-ichi NPP varied from the nearest one (Hirono) to the farthest one (Tsukuba) (Figure 1C). The ground radiation dose at the time of butterfly sampling also varied from Hirono to Tsukuba (Figure 1D), but the highest locality was Motomiya, as expected from the dispersion of radioactive materials to the northwestern area from the Fukushima Dai-ichi NPP (Chino et al. 2011; Kinoshita et al. 2011; Hirose 2012; Torii et al. 2013; Endo et al. 2015).

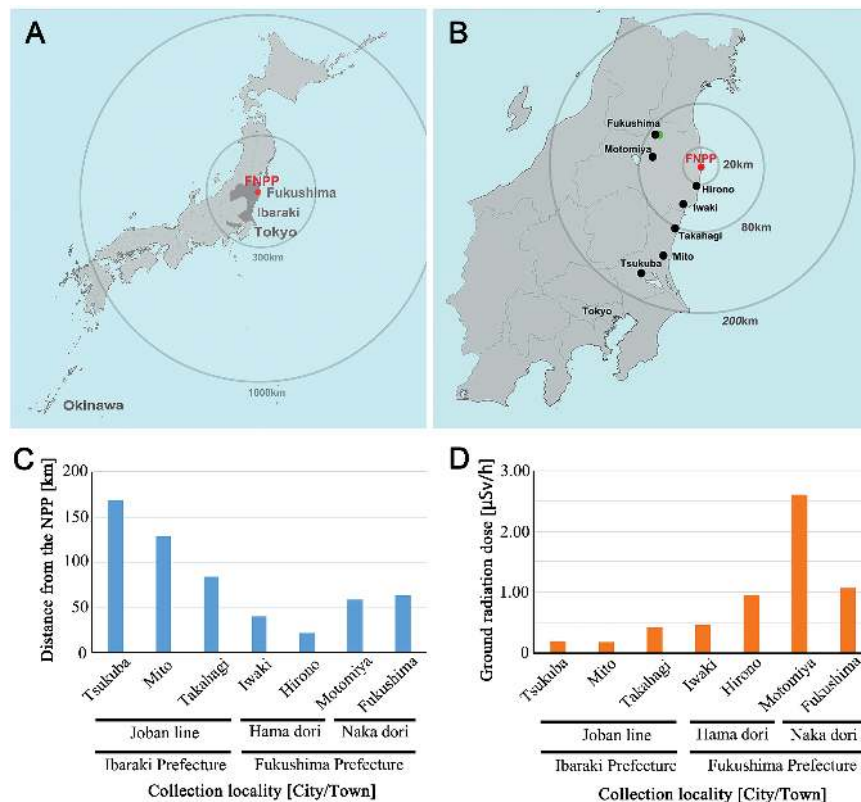


Figure 1. Geographical locations of collection localities and 2 factors that characterize them. (A) Location of the Fukushima Dai-ichi NPP (FNPP) in Japan. Fukushima Prefecture, Ibaraki Prefecture and the Tokyo Metropolitan Area are shaded. Circles indicate distance from the Fukushima Dai-ichi NPP. (B) Seven collection localities for butterfly samples. Green dot in Fukushima City indicates the collection locality for the host plant leaves. Circles indicate distance from the Fukushima Dai-ichi NPP. (C) Distance from the Fukushima Dai-ichi NPP. (D) Ground radiation dose. Motomiya is the most contaminated locality, followed by Fukushima and Hirono. See online version for full color.

Robustness of the Local Populations

We first examined the degrees of health of the local populations. Larvae were obtained from females that were caught from the 7 localities in May 2011 (Spring 2011) and reared under the standard conditions using non-contaminated leaves from Okinawa without artificial radiation exposure. The survival curves indicate that the survival rate at the larval and pupal stages was relatively low in all localities (Figure 2A), considering that the survival rate of non-polluted populations (e.g., Okinawa populations) throughout the developmental stages are usually more than 90% (Hiyama et al. 2012a; Taira et al. 2014; Nohara et al. 2014b). The adult survival rates varied but were highest in Tsukuba among the 7 localities. This result was expected because Tsukuba is the least contaminated locality among the surveyed.

The adult individuals that successfully eclosed were visually examined for morphological abnormalities under a stereomicroscope. The number of morphologically normal adults, with respect to the number of starting larvae, was used to obtain the normality rate of natural exposure and the robustness (Figure 2B). In Ibraki Prefecture (i.e., Tsukuba, Mito, and Takahagi), the robustness value decreased with the increase in the pollution level, suggesting the population-level damage in these localities. However, the population of Motomiya, which was the most polluted locality among the surveyed localities, had the second highest level of robustness. This result is consistent with the idea that the Motomiya population evolved to be normal in the highly polluted environment. There was no clear relationship between the robustness value and the distance from the Fukushima Dai-ichi NPP ($\rho = -0.37$, $P = 0.50$) or between the robustness value and the ground radiation dose ($\rho = 0.60$, $P = 0.24$).

For reference, we obtained the normality rates of May 2011 (Spring 2011) and September/October 2011 (Fall 2011) based on the total abnormality rates in Hiyama et al. (2012a) and examined

their dynamics in 7 localities over time (Figure 2C). Differences of the normality rates between Spring 2012 and Fall 2011 were also calculated (Figure 2D). In Hirono and Motomiya, 2 highly contaminated localities, the normality rate differences were highly positive, suggesting that these populations regained normality in this period.

Resistance to External Irradiation

A randomly allocated portion of the offspring larvae was similarly reared, but this time, larvae were subjected to external radiation exposure from a ^{137}Cs radiation source. The range of cumulative radiation dose for a randomly allocated larva was from 7.85 mSv to 124.26 mSv. Survival curves indicated low survival rate (i.e., high mortality) in most localities, especially at the larval stage except for the Hirono population (Figure 3A). For the Hirono population, relatively high survival rate (low mortality) was observed throughout all stages among the 7 localities.

We visually examined morphological abnormalities of adult individuals that eclosed successfully. Based on these abnormal individuals identified, we obtained the number of morphologically normal adults, which was subsequently divided by the number of starting larvae to obtain the normality rate of external exposure. This normality rate of external exposure was correlated with the normality rate of natural exposure with the exception of the Hirono sample ($\rho = 0.80$, $P = 0.11$ excluding the Hirono sample as an outlier) (Figure 3B). Although this relationship was not statistically significant, this could be overcome by an increase of sampling localities. If so, these results suggest that radiation resistance (or susceptibility) to external irradiation may reflect general state of health.

We here calculated the value of resistance to external irradiation (R_{ex}) (Figure 3C). As expected from the aforementioned survival curve, the Hirono population showed the highest resistance; it scored more than 100%. The populations of Motomiya and Fukushima,

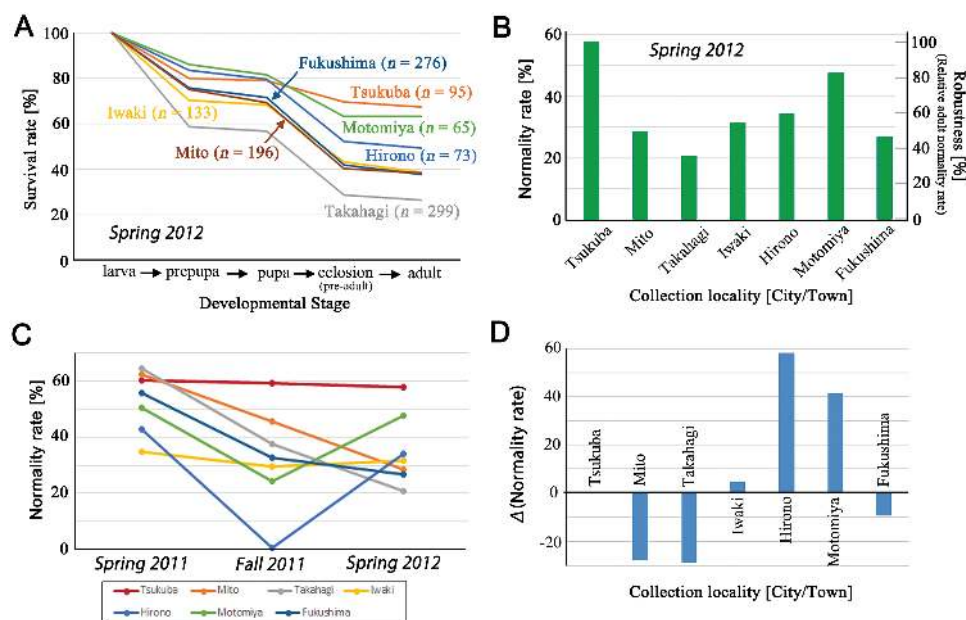


Figure 2. Characterization of local populations of the pale grass blue butterfly. Larvae were reared with non-contaminated leaves under the standard conditions. (A) Survival curve. The numbers of starting larvae are indicated as n . (B) Normality rate (left y-axis) and robustness (right y-axis). To obtain the robustness value, the Tsukuba population, which is the least contaminated one, is set at 100%. After the Tsukuba population, the Motomiya population shows the highest value. (C) Dynamics of the normality rates from Spring 2011 to Spring 2012. Original data were presented as the total abnormality rates in Hiyama et al. (2012a). Hirono and Motomiya show v-shaped dynamics, indicating a decrease and then an increase of the normality rate. (D) Normality difference. It was calculated as a subtraction of Fall 2011 from Spring 2012. Hirono and Motomiya show highly positive values. See online version for full color.

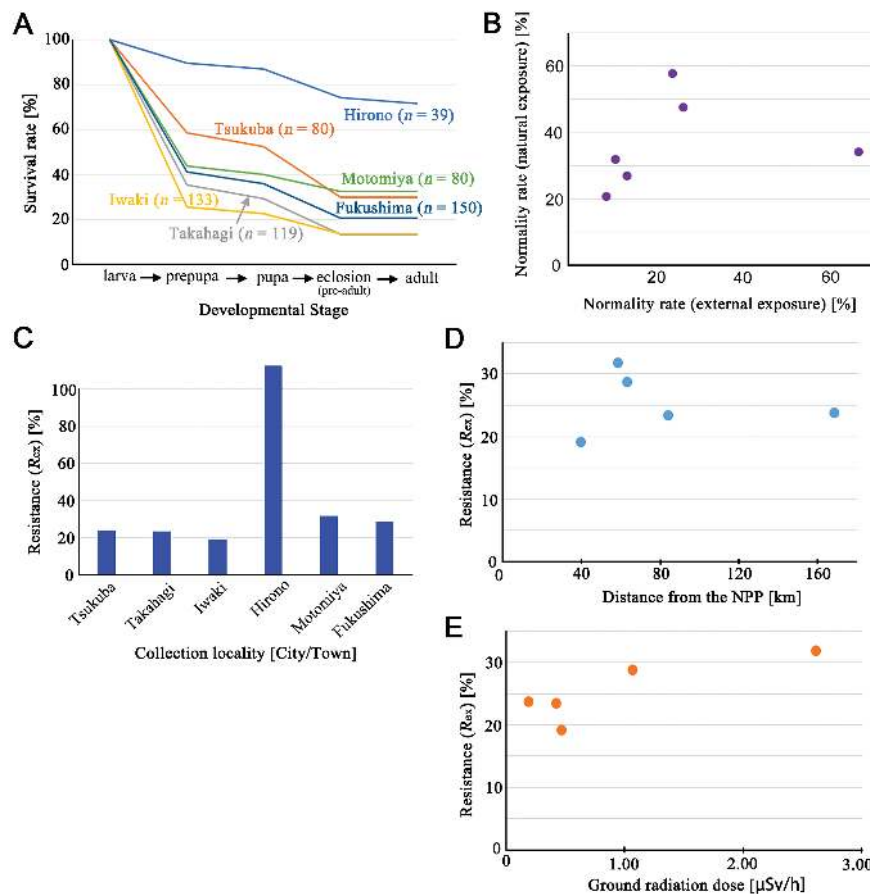


Figure 3. External exposure experiment. Larvae were reared with non-contaminated leaves under the standard conditions in addition to external exposure to γ -irradiation from ^{137}Cs . **(A)** Survival curve. The numbers of starting larvae are indicated as n . **(B)** Scatter plot of normality rates. Hirono was plotted here but was excluded as an outlier to calculate correlation coefficient. **(C)** Resistance to external exposure (R_{ex}). **(D)** Scatter plot of R_{ex} versus distance from the Fukushima Dai-ichi NPP. Hirono was excluded as an outlier. **(E)** Scatter plot of R_{ex} versus the ground radiation dose. Hirono was excluded as an outlier. See online version for full color.

which were heavily contaminated localities, showed higher values than the populations of Iwaki, Takahagi, and Tsukuba, which were less contaminated localities.

The possible relationships of R_{ex} with the distance from the Fukushima Dai-ichi NPP and with the ground radiation dose were examined, but we obtained no significant correlation coefficients in either case ($\rho = -0.37$, $P = 0.50$ for distance and $\rho = 0.60$, $P = 0.24$ for radiation dose). Even when the exceptionally high Hirono population was excluded as an outlier (Figure 3D, E), correlation coefficient for R_{ex} with the distance from the Fukushima Dai-ichi NPP ($\rho = 0.10$, $P = 0.95$) and that with the ground radiation dose ($\rho = 0.60$, $P = 0.35$) were not statistically significant.

Resistance to Internal Irradiation

Another randomly allocated portion of the offspring larvae was similarly reared but without external radiation exposure. Instead, they were reared with contaminated leaves that were collected from Watari, Fukushima City. The levels of contamination were: 93.0 ± 0.6 Bq/kg for ^{137}Cs and 65.4 ± 0.6 Bq/kg for ^{134}Cs , which yielded 158.4 ± 0.9 Bq/kg in total at the time of larval rearing.

The survival curves indicate that the Motomiya population had the highest survival rate, whereas the Takahagi population had the lowest survival rate (Figure 4A). Similarly to the external exposure experiment, the survival rate was low at the larval and pupal stages.

However, the Motomiya, Tsukuba, and Hirono populations showed relatively small changes throughout the stages, and the populations of Fukushima, Iwaki, and Mito behaved similarly.

We visually examined the morphological abnormalities of adults that successfully eclosed, and we obtained the number of morphologically normal individuals, yielding the normality rate of internal exposure. This normality rate of internal exposure was highly correlated with the normality rate of natural exposure ($\rho = 0.93$, $P = 0.023$) (Figure 4B). These results suggest again that radiation resistance (or susceptibility) to internal irradiation reflects general state of health.

We obtained the value of resistance to the internal irradiation (R_{in}) (Figure 4C) and examined the possible relationships of the R_{in} values with the distance from the Fukushima Dai-ichi NPP (Figure 4D) and with the ground radiation dose (Figure 4E). We obtained Spearman correlation coefficients for R_{in} with the distance from the Fukushima Dai-ichi NPP ($\rho = -0.79$, $P = 0.048$) and with the ground radiation dose ($\rho = 0.75$, $P = 0.066$). Both correlation coefficients were reasonably large in absolute numbers, although their P -values were not very small.

Discussion

Significance of the Experimental Outputs

In the previous study, we showed dynamic changes of the morphological abnormality rates and mortality rates in 3 years from

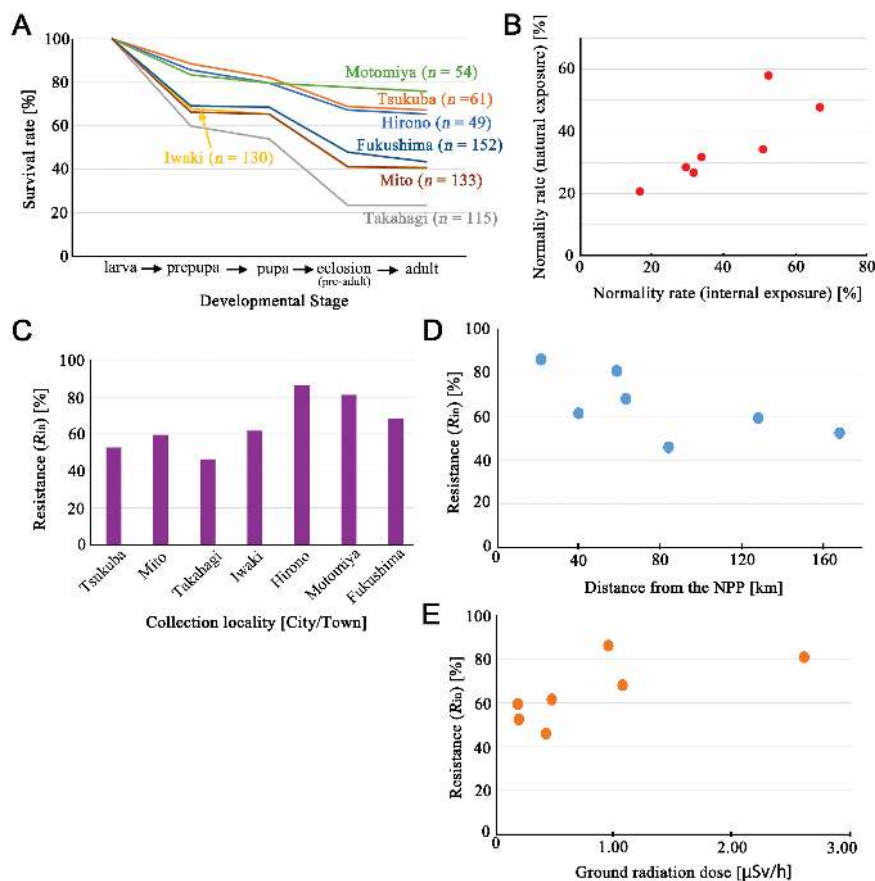


Figure 4. Internal exposure experiment. Larvae were reared under the standard conditions, with the exception of feeding with contaminated leaves from Watari, Fukushima City. (A) Survival curve. The numbers of starting larvae are indicated as n . (B) Scatter plot of normality rates. (C) Resistance to internal exposure (R_{in}). (D) Scatter plot of R_{in} versus the distance from the Fukushima Dai-ichi NPP. (E) Scatter plot of R_{in} versus the ground radiation dose. See online version for full color.

immediately after the Fukushima nuclear accident (Hiyama et al. 2015). An increase of the normality rate in Spring 2012 immediately after an decrease in Fall 2011 was observed in the high-radiation localities. In contrast, a decrease of the normality rate seem to continue in the low-radiation localities. Thus, we reasoned that evolutionary velocity of regaining normality (and, as a result, the degrees of health) could vary among the populations, depending on the contamination levels.

In this study, we demonstrated that the degrees of health, represented by the normality rates and the robustness values, vary among the populations under the natural exposure conditions as of May 2012 (Spring 2012). It is important to note that the individuals that were reared under the laboratory conditions had no exposure of external or internal artificial irradiation, although their parents and ancestors were certainly exposed to the artificial radionuclides from the Fukushima Dai-ichi NPP. Because the rearing conditions for the offspring generation in the laboratory were all the same among the individuals from different localities, what we observed here is locality-dependent heritable effects.

These heritable effects include not only population-level genetic effects that may have been shaped through natural selection but also epigenetic effects from locality-dependent environments (Mousseau and Dingle 1991; Mousseau and Fox 1998). The maternal effects are known to contribute to the offspring fitness; the maternal effects are epigenetic but their phenotypes are subjected to selection, leading to

genetic changes at the population level (Mousseau and Dingle 1991; Mousseau and Fox 1998). Paternal effects may also contribute to epigenetic inheritance of environmental information (Skinner et al. 2015; Soubry 2015). Long-term low-dose exposure of ionizing radiation could induce genomic instability, which is transgenerational (Merrifield and Kovalchuk 2013). Because irradiation from radioactive pollutants continues generation after generation, genomic instability would be induced in every generation by the chronic exposure, resulting in its accumulation over time. Therefore, in the present study, synthetic local influences including both genetic and epigenetic ones are likely reflected in the normality rates and the robustness values for each locality. Our experimental results do not reveal these mechanisms but simply are consequences of these unknown mechanisms.

The normality rates and robustness values that were obtained in this study do not indicate fitness and fecundity of the “normal” individuals that eclosed successfully under the laboratory conditions. Fitness and fecundity may vary even among the “normal” individuals. We did not test this point. Rather, in this study, we focused on deaths and morphological abnormalities that considerably decrease fitness of individuals, and we obtained the normality rates (and robustness) of populations (not individuals). Our experimental systems are thus not intended to demonstrate natural selection operating in the field, although our results suggest the possibility.

Many butterfly species are known to use various host plants, which is also known to be a source of the maternal effects that influence the offspring fitness (Mousseau and Dingle 1991; Mousseau and Fox 1998). Because the pale grass blue butterfly is monophagous, the possible effects from the host plant variation can be ignored entirely in this species. However, it is known that maternal diet quality determines the offspring's stress response and fitness (Mousseau and Dingle 1991; Mousseau and Fox 1998). Maternal diet quality certainly varied in the present study, because the maternal host plant in each locality is contaminated by artificial radionuclides at various levels, depending on the collection localities of the females. In contrast, diet quality for larvae that were reared in the laboratory did not vary, because the host plant leaves were collected from a single source, Nishihara Town, Okinawa, for our rearing experiments, except for the internal exposure experiment, in which case the leaves were collected from Watari, Fukushima City. Thus, it is likely that our results of the offspring generation from the field-caught females partly reflect maternal diet quality of the parent generation.

It is to be understood that phenotypic plasticity of the pale grass blue butterfly is mainly observed in clinal size changes that are geographical as well as temporal (Taira et al. 2015b). Additionally, temperature-shock-induced color pattern modifications (not seasonal polyphenism) are known (Buckley et al. 2010; Otaki et al. 2010; Hiyama et al. 2012b). These plastic traits of this butterfly species are unlikely to be significant in the present study, because all female butterfly samples were collected within a reasonably small geographic range and within a reasonably short time span.

Locality-Dependence of Robustness

We found that in Ibraki Prefecture (i.e., Tsukuba, Mito, and Takahagi), the robustness value decreased with the increase in the pollution level, which suggests that these populations might have been deteriorating and subjected to natural selection 1 year after the Fukushima nuclear accident. Interestingly, the Motomiya and Hirono populations, which were heavily polluted, had the robustness values of approximately 80% and 60%, respectively. In other words, the robustness values (and the survival rates) were high not only in the least contaminated locality (i.e., Tsukuba) but also in the most contaminated localities (i.e., Motomiya and Hirono). These high robustness values in the highly polluted localities can be explained by the faster development of radiation resistance in more polluted areas than in less polluted areas. That is, the robustness results suggest that radiation resistance has evolved relatively quickly in the Motomiya and Hirono populations. However, unknown causes for this radiation resistance, other than the Fukushima nuclear accident, cannot be excluded.

Here we discuss 3 points to be considered in interpreting our results in this study. First, we cannot rule out the possibility that the locality-dependent heritable effects, which were observed as the normality rates and robustness of the offspring generation, may stem from locality-dependent environmental factors other than radioactive contamination. This possibility cannot be overlooked, but it is true that we collected female butterflies from geographically similar localities (all in Fukushima and Ibaraki Prefectures) within a reasonable time frame.

Second, we assume that before the Fukushima nuclear accident, all local populations were equally highly vulnerable to ionizing radiation at least at the level of the Tsukuba population. We believe that this assumption is correct, based on our own studies (Hiyama et al. 2012a, 2015). Especially important is the external

and internal irradiation experiments that were performed using the Okinawa population (Hiyama et al. 2012a). Okinawa is the least contaminated locality in Japan (because it is the farthest from the Fukushima Dai-ichi NPP), and thus the Okinawa population can be considered to retain the pre-accidental state of this butterfly even after the Fukushima nuclear accident. The Okinawa population was highly vulnerable to external and internal irradiation despite its high normality rate (Hiyama et al. 2012a), showing low resistance values (R_{ex} and R_m). Therefore, it is reasonable to assume that the 7 local populations that were surveyed in the present study equally had low resistance values before the Fukushima nuclear accident.

Third, the abnormality rate of the Hirono population in the fall of 2013 was relatively low (see Figure 8b in Hiyama et al. 2015), being consistent with the present study. However, the level of the Motomiya population was relatively high (see Figure 8b in Hiyama et al. 2015), being inconsistent with the present study. Because the Hirono population is unique but the Motomiya population is not in the external exposure experiment, adaptive evolution may be more prominent in Hirono than in Motomiya. Alternatively, unknown locality-dependent confounding factors might have existed to differentiate these 2 local populations.

Correlation Between Robustness and Resistance

A simple but potentially important finding of this study is that the normality rate of the external or internal exposure experiment is reasonably correlated with that of natural exposure. This finding may not be surprising, but these normality rates do not have to be correlated with each other if the resistance that evolved is specific to irradiation. Because the normality rate of natural exposure is a simple indication of the health state of the population, these correlations mean that the radiation resistance to external or internal irradiation can be considered a reflection of general health state. The radiation resistance we detected is thus likely related with general stress response. The heritable radiation resistance does not seem to have immediate fitness cost under the laboratory conditions.

External and Internal Irradiation Experiments

In our irradiation system, different larvae even in a given group might have received different doses. In the external exposure experiment, an exact dose for a larva depends on where a larva was located with respect to the radiation source. In the internal exposure experiment, the contamination level of leaves may vary from leaf to leaf. This kind of heterogeneity of irradiation may be unavoidable but does not undermine our experimental results.

Previously, we performed similar external and internal experiments using the pale grass blue butterfly from the least contaminated area, Okinawa, and we obtained a dose-dependent decrease in survival rate in both external and internal exposure experiments (Hiyama et al. 2012a). The results of the present study strongly contrast with these previous results in that the populations of higher pollution levels such as Hirono and Motomiya showed higher survival and resistance. Considering that the butterfly populations are unlikely to have high radiation resistance (or high ability of general stress response) before the accident based on the high morphological abnormality and mortality rates in Fall 2011 (Hiyama et al. 2012a, 2015), the resistance that we detected here likely evolved recently after the nuclear accident.

The highest R_m was observed in the Hirono population (a score of nearly 90%), and the populations of Motomiya and Fukushima showed high R_m values, compared with those of other localities. In

these points, R_m values were different from R_{ex} values. Spearman correlation coefficient between R_{ex} and R_m ($\rho = 0.83$, $P = 0.064$) was not statistically significant. Overall, we conclude that the external and internal exposure experiments had different results in terms of the locality distribution patterns of resistance values. The mechanisms of resistance (or susceptibility) to the external and internal exposures may be somewhat different, and these mechanisms might have independently evolved in the butterfly, considering the difference between R_{ex} and R_m . However, this difference may simply be because of different exposure levels in our external and internal experiments.

The external resistance R_{ex} was notably high in the Hirono population but generally small in other populations. We do not know why the Hirono population was so exceptionally resistant to external irradiation. It may simply be a stochastic sampling error. However, as shown in Figure 1C and also in Hiyama et al. (2015), the Hirono population of this butterfly experienced severe deterioration in Fall 2011. Selection pressure in Hirono was probably much higher than that in other localities examined in this study. In fact, Hirono is the locality closest to the Fukushima Dai-ichi NPP among the surveyed localities; it is located to the immediate south of the Fukushima Dai-ichi NPP (nearly 20 km away from the Fukushima Dai-ichi NPP). Although most radiation plumes from the Fukushima Dai-ichi NPP were dispersed toward areas northwest of the Fukushima Dai-ichi NPP, early plumes that contained ^{131}I , $^{129\text{m}}\text{Te}$ and other short-lived radionuclides were circularly distributed from the Fukushima Dai-ichi NPP (Torii et al. 2013; Endo et al. 2015). Their β -ray effects on animals would never be negligible (Endo et al. 2014). Thus, the effect of the early plumes on the Hirono population was likely exceptionally large. We speculate that the early effects were almost exclusively mediated by external exposure, particularly from short-lived radionuclides, because the overwintering larvae in March hardly eat leaves. This external radiation exposure immediately after the explosion of the Fukushima Dai-ichi NPP probably contributed to the high R_{ex} of the Hirono population. This speculation is consistent with the fact that the Hirono population behaved differently from other local populations regarding the mortality and abnormality rates of the offspring generation in the fall of 2011, which were more than 90% (Hiyama et al. 2012a).

In the internal exposure experiment, we obtained reasonably high correlation coefficients between resistance and distance from the Fukushima Dai-ichi NPP. One possible interpretation is that local populations of the pale grass blue butterfly in the highly contaminated areas were more resistant to irradiation than in the less contaminated areas. A similar case has been reported in Chernobyl (Galván et al. 2014). Because the butterfly populations that were used in the present study were the 7th generation after the Fukushima nuclear accident, an adaptive evolution process might have been triggered readily after the accident, and the process has still been under way.

Technically, combinations of the external and internal exposures in the laboratory would reproduce the field conditions more faithfully, which will help to understand not only the realistic situation of larvae in the field but also combinatorial effects of the 2 modes of exposures in reference to the present results.

Evolutionary Changes of Robustness and Resistance

The present results suggest a causal involvement of the Fukushima nuclear accident in the evolutionary changes of robustness and radiation resistance. We cannot prove the causality for the high radiation

resistance in this study, and we cannot specify a specific causal substance within the leaf samples in this study alone. However, we have already demonstrated high toxicity of the leaves from the polluted areas when ingested (Hiyama et al. 2012a; Nohara et al. 2014a, 2014b; Taira et al. 2015a). Because the larvae at the contaminated areas are forced to eat contaminated leaves, using the field-collected samples in the laboratory is the best experimental strategy to investigate what has occurred in the field.

Our results suggest the evolution of radiation resistance after several generations, which is approximately a year after the Fukushima accident, in the pale grass blue butterfly. In a previous study, we observed the real-time evolution of cold resistance and color pattern changes in the same species in a few years, and we reproduced this field case in the artificial selection experiment using the same species (Otaki et al. 2010). Reasonable color pattern changes were observed from the 6th generation onward (Otaki et al. 2010). When the selection pressure is high, evolution may be reasonably rapid, at least in this species. Adaptation to radiation stress in naturally high-radiation areas has been reported in *Drosophila* (Kratz 1975) and other organisms (Møller and Mousseau 2013a). Our study is an interesting and important case that describes the evolution of a physiologically important function in the field, which was likely triggered by anthropogenic mutagenic substances. Future studies should monitor the changes that have occurred in the polluted butterfly populations.

Funding

This work was supported by the Kondo Jiro Grant for Environmental Research from The Asahi Glass Foundation, Tokyo, and by donations from the general public.

Acknowledgments

The authors thank S. Gima, A. Tanahara, S. Kinjo, Y. Ohno, M. Iwasaki, and M. Iwata for the technical assistance; J. Ishida, K. Yoshida, N. Itoh, K. Nakanome, and J. Nohara for collecting host plant leaves; and members of the BCPH Unit of Molecular Physiology for the technical assistance and discussion. The authors also greatly appreciate donors who philosophically and financially supported our Fukushima Project. Data availability: All primary data were presented in this paper.

References

- 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.
- Barton NH, Briggs DEG, Eisen JA, Goldstein DB, Patel NH. 2007. *Evolution*. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
- Bonisolli-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.
- Chino M, Nakayama H, Nagai H, Terada H, Katata G, Yamazawa H. 2011. Preliminary estimation of release amount of ^{131}I and ^{137}Cs accidentally discharged from the Fukushima Daiichi nuclear power plant into the atmosphere. *J. Nucl. Sci. Technol.* 48:1129–1134.
- Cook LM. 2003. The rise and fall of the *Carbonaria* form of the peppered moth. *Q Rev Biol.* 78:399–417.
- Cook LM, Grant BS, Saccheri IJ, Mallet J. 2012. Selective bird predation on the peppered moth: the last experiment of Michael Majerus. *Biol Lett.* 8:609–612.

- Cook LM, Saccheri IJ. 2013. The peppered moth and industrial melanism: evolution of a natural selection case study. *Heredity (Edinb)*. 110:207–212.
- Ehrlich PR. 2003. Butterflies, test systems, and biodiversity. In: Boggs CL, Watt WB, Ehrlich PR, editors. *Butterflies—Ecology and evolution taking flight*. Chicago (IL): The University of Chicago Press. p. 1–6.
- Ellegren H, Lindgren G, Primmer CR, Møller AP. 1997. Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature*. 389:593–596.
- Endo S, Kajimoto T, Tanaka K, Nguyen TT, Hayashi G, Imanaka T. 2015. Mapping of the cumulative β -ray dose on the ground surface surrounding the Fukushima area. *J Radiat Res*. 56(suppl 1):i48–i55.
- 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(3):476–483.
- Futuyma DJ. 2013. *Evolution*. 3rd ed. Sunderland (MA): Sinauer Associates.
- Galván I, Bonisoli-Alquati A, Jenkinson S, Ghanem G, Wakamatsu K, Mousseau TA, Møller AP. 2014. Chronic exposure to low-dose radiation at Chernobyl favors adaptation to oxidative stress in birds. *Funct. Ecol*. 28:1387–1403.
- 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.
- Hillis DM, Sadava D, Heller HC, Price MV. 2012. *Principles of life*. Sunderland (MA): Sinauer Associates.
- Hirose K. 2012. 2011 Fukushima Dai-ichi nuclear power plant accident: summary of regional radioactive deposition monitoring results. *J Environ Radioact*. 111:13–17.
- 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, 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.
- Hiyama A, Taira W, Otaki JM. 2012b. Color-pattern evolution in response to environmental stress in butterflies. *Front Genet*. 3:15.
- Horiguchi T, Yoshii H, Mizuno S, Shiraiishi 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.
- 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.
- Kinoshita N, Sueki K, Sasa K, Kitagawa J, Ikarashi S, Nishimura T, Wong YS, Satou Y, Handa K, Takahashi T, et al. 2011. Assessment of individual radionuclide distributions from the Fukushima nuclear accident covering central-east Japan. *Proc Natl Acad Sci U S A*. 108:19526–19529.
- Kratz FL. 1975. Radioresistance in natural populations of *Drosophila nebulosa* from a Brazilian area of high background radiation. *Mutat Res*. 27:347–355.
- Majerus MEN. 1998. *Melanism: evolution in action*. Oxford (UK): Oxford University Press.
- Merrifield M, Kovalchuk O. 2013. Epigenetics in radiation biology: a new research frontier. *Front Genet*. 4:40.
- 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. Strong effects of ionizing radiation from Chernobyl on mutation rates. *Sci Rep*. 5:8363.
- Møller AP, Nishiumi I, Suzuki H, Ueda K, Mousseau TA. 2013. Differences in effects of radiation on abundance of animals in Fukushima and Chernobyl. *Ecol Indic*. 24:75–81.
- Mousseau TA, Dingle H. 1991. Maternal effects in insect life histories. *Annu Rev Entomol*. 36:511–534.
- Mousseau TA, Fox CW. 1998. The adaptive significance of maternal effects. *Trends Ecol Evol*. 13:403–407.
- Muller HJ. 1927. Artificial transmission of the gene. *Science*. 66:84–87.
- Muller HJ. 1928. The production of mutations by X-Rays. *Proc Natl Acad Sci U S A*. 14:714–726.
- Murase K, Murase J, Horie R, Endo K. 2015. Effects of the Fukushima Daiichi nuclear accident on goshawk reproduction. *Sci Rep*. 5:9405.
- Nadeau NJ, Pardo-Diaz C, Whibley A, Supple MA, Saenko SV, Wallbank RW, Wu GC, Maroja L, Ferguson L, Hanly JJ, et al. 2016. The gene *cortex* controls mimicry and crypsis in butterflies and moths. *Nature*. 534:106–110.
- Nijhout HF. 1991. *The development and evolution of butterfly wing patterns*. Washington (DC): Smithsonian Institution Press.
- 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.
- Oda H, Kitazoe N. 2002. *Observation encyclopedia of lycaenid butterflies*. Tokyo (Japan): Kaisei-sha.
- 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.
- Pacini F, Vorontsova T, Demidchik EP, Molinaro E, Agate L, Romei C, Shavrova E, Cherstvoy ED, Ivashkevitch Y, Kuchinskaya E, et al. 1997. Post-Chernobyl thyroid carcinoma in Belarus children and adolescents: comparison with naturally occurring thyroid carcinoma in Italy and France. *J. Clinical Epidemiol. Metab*. 82:3563–3569.
- Shirai Y, Takahashi M. 2005. Effects of transgenic Bt corn pollen on a non-target lycaenid butterfly, *Pseudaizeeria maha*. *Applied Entomol. Zool*. 40:151–159.
- Shirōzu T. 2006. *The standard of butterflies in Japan*. Tokyo (Japan): Gakken.
- Skinner MK, Guerrero-Bosagna C, Haque MM. 2015. Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics*. 10:762–771.
- Soubry A. 2015. Epigenetic inheritance and evolution: A paternal perspective on dietary influences. *Prog Biophys Mol Biol*. 118:79–85.
- 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–i18.
- 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.

- Torii T, Sugita T, Okada CE, Reed MS, Blumenthal DJ. 2013. Enhanced analysis methods to derive the spatial distribution of ^{131}I deposition on the ground by airborne surveys at an early stage after the Fukushima Daiichi nuclear power plant accident. *Health Phys.* 105:192–200.
- van't Hof AE, Edmonds N, Dalíková M, Marec F, Saccheri IJ. 2011. Industrial melanism in British peppered moths has a singular and recent mutational origin. *Science.* 332:958–960.
- Van't Hof AE, Campagne P, Rigden DJ, Yung CJ, Lingley J, Quail MA, Hall N, Darby AC, Saccheri IJ. 2016. The industrial melanism mutation in British peppered moths is a transposable element. *Nature.* 534:102–105.
- Washitani I, Yoshioka A, Suda S, Yasukawa M, Kitsuregawa M. 2013. The pale grass blue butterfly in the citizen science Tokyo butterfly monitoring project. *Kagaku.* 83:0961–0966.
- 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.
- Wickler W. 1968. *Mimicry in plants and animals*. London (UK): World University Library.
- Wolt JD, Conlan CA, Majima K. 2015. An ecological risk assessment of Cry1F maize pollen impact to pale grass blue butterfly. *Environ Biosafety Res.* 4:243–251.
- Yata O. 2007. *Iconographia Insectorum Japonicorum Colore Naturali Esita*. Vol. I. Tokyo (Japan): Hokuryukan.
- Zimmer C, Emlen DJ. 2013. *Evolution: making sense of life*. Greenwood Village (CO): Roberts and Company.