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Morphological Abnormalities in True Bugs (*Heteroptera*) near Swiss Nuclear Power Stations

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After the nuclear accidents of Chernobyl and Fukushima, several studies reported adverse health effects on wildlife animals. Epidemiological studies in humans found significant increases of leukemia rates in young children residing within 5 km from nuclear power plants. This study investigates morphological abnormalities in true bugs (*Heteroptera*), collected in the environs of three Swiss nuclear power stations (NPS). The objective of the study is to test whether there is an increased frequency of abnormalities in the vicinity of NPS. We found a frequency of abnormalities of 14.1% at distances $r < 5$ km and a frequency of 6.8% for distances $r > 5$ km, a rate ratio of 2.1 ($P < 0.0001$). The corresponding odds ratio was 2.26 (95% CI: 1.59, 3.18). We also conducted logistic regression of abnormality rates on reciprocal distance for each NPS site. The trend was significant for NPS Beznau (regression coefficient $\beta = 1.5 \pm 0.3$, $P < 0.0001$) but not significant for NPS Gösgen und NPS Leibstadt with little samples within 5 km. To the best of our knowledge, this study is the first to find adverse health effects on insects near operating nuclear power plants. Due to its ecological design, however, it cannot answer the question whether the effect is caused by radiation from nuclear power plants.

Keywords: insects, abnormalities, radiation, nuclear power station, NPS, logistic regression.

Introduction

After the Chernobyl nuclear disaster in 1986, studies of possible adverse radiation effects on biota were conducted in contaminated regions of Ukraine, Belarus, Sweden, and other countries. In the Chernobyl exclusion zone, irradiated populations of plants and animals exhibited a variety of morphological abnormalities and mutations that were rare prior to 1986.[1] Møller and Mousseau conducted a meta-analysis to examine the relationship between radiation and mutation rates in Chernobyl across 45 published studies, covering 30 species.[2] The overall effect of radiation on mutation rates was very large and highly statistically significant.

After the triple meltdown at the Fukushima Daiichi nuclear power plant in March 2011, similar studies were conducted in Japan. In a review article, Aliyu et al. discussed the outcomes of studies on plants and animals in the forests of Fukushima region.[3] A range of physiological, developmental, morphological, and behavioral consequences of exposure to radioactivity were recorded. Some of the effects observed in the exposed populations include hematological aberrations in Fukushima monkeys; genetic, developmental, and morphological aberrations in butterflies; declines in abundances of birds, butterflies, and cicadas; and morphological abnormalities in insects. As an example, Akimoto investigated morphological abnormalities in gall-forming aphids (*Hemiptera*) in a radiation-contaminated area of Fukushima Prefecture.[4] The proportion of abnormalities was significantly higher in Fukushima than in the control areas; of 164 aphids from *Tetraneura sorinigalls* collected 32 km from the Fukushima Daiichi NPS in spring 2012, 13.2% exhibited morphological abnormalities. In contrast, in seven control areas, aphids with abnormal morphology accounted for only 3.8% on an average.

Epidemiologic studies of leukemia in children living near nuclear power stations (NPS) were spurred by the discovery of a cluster of childhood leukemia near the nuclear reprocessing facility Sellafield in the UK in the 1980s. Increased leukemia rates in young children living near (< 5 km) nuclear power stations were found in Germany,[5][6] Great Britain,[7] Switzerland,[8] and France.[9] An analysis of the pooled data from these four countries found a significant 45% increase of leukemia in children under five in the 5 km zone when compared with distances > 5 km.[10]

Since 1969, Hesse-Honegger had collected true bugs (*Heteroptera*) in Switzerland and other countries to study and paint variety of species.[11] After the accident at the nuclear power plant Chernobyl 1986, in 1987 she started collecting and investigating true bugs in fallout areas of Chernobyl. In 1988, she began her research in the vicinity nuclear accident areas like Chernobyl, Three Mile Island, and Fukushima, as well as nuclear facilities like Sellafield, NPS Krümmel, or Hanford. She collected over 18 000 true bugs leafhoppers (Auchenorrhyncha) and ladybird beetles (Coccinellidae). They were subjected to detailed visual inspection. An increased frequency of abnormalities was noticed close to nuclear power plants in Switzerland (Canton Aargau), France (nuclear reprocessing plant La Hague), and Germany (NPS Gundremmingen). The frequencies of morphological aberrations exceeded 20%, which is much higher occurrence than usually observed. Her studies included reference biotopes in Switzerland, Ireland, and Vietnam.[11]

This study uses data provided by Hesse-Honegger to test whether the rise of morphological abnormalities in the vicinity of Swiss nuclear power stations is statistically significant. It encompasses three field studies conducted between 1992 and 2013. The first study of insects around Swiss nuclear power plants was conducted in 1987. As the number of insects per research point was below 65, they were not included in this study. For the Aargau-study she decided to collect 65 true bugs per research point, which was sufficient to detect enough malformations but not enough to deplete a population on a given research location. For the Entlebuch-study, she also collected 65 true bugs per research point.

Results and Discussion

We found a significantly increased frequency of abnormalities within 5 km of the three nuclear power stations relative to frequencies at distances > 5 km. The estimate of the odds ratio is 2.26 (95% CI: 1.59, 3.18), $P < 0.001$. The mean frequency of abnormalities is estimated at 14.1% for distances $r < 5$ km and 6.8% for $r > 5$ km, a relative rate of 2.1. The data show large variability; the deviance is 150.0 with 73 degrees of freedom. With an alternative cut point of 10 km, the odds ratio of abnormalities in true bugs decreases to 1.83 (1.30, 2.54), $P < 0.001$.

To test the distance dependency at each individual NPS site, we excluded samples within 5 km of the remaining two NPS sites. Around every NPS site, abnormality rates increase with proximity to the reactor. The trend parameter β was highly significant for NPS Beznau ($\beta = 1.5 \pm 0.3$, $P < 0.001$) but, due to sparse data, was not significant for NPS Leibstadt and NPS Gösgen (there was only one sample near ($r < 5$ km) NPS Leibstadt and two samples near NPS Gösgen, but six samples near NPS Beznau). The trend of abnormalities in the environs of NPS Beznau is shown in *Figure 1*.

A major weakness of our study is the unblinded analysis of the bug samples for abnormalities, i.e., the fact that the same person collected and inspected the bugs. A possible bias can therefore not be excluded. Also, too little samples were taken in the 5 km zones of NPS Leibstadt and NPS Gösgen, and no samples were collected near the fourth nuclear power station Mühleberg.

After Fukushima, a study of morphological abnormalities in aphids determined a frequency of abnormalities of 13.2% in contaminated territories near the Fukushima Daiichi NPS and of 3.8% in control regions of Japan.[4] But one year after the Fukushima accident, radiation dose levels in contaminated areas were 10 – 100-times above background while, according to official estimates, the annual dose to the population living near nuclear power stations is < 0.01 mSv, i.e., two orders of magnitude less than the dose from natural background radiation.[12] The fact that we find a similar increase of abnormalities in true bugs near normally operating nuclear power plants as reported for aphids in[4] is unexpected and calls for an explanation. However, our result for the effect size agrees with the twofold increase of leukaemia in young children near German NPS which is also unexplained.

Conclusions

To the best of our knowledge, our study is the first to investigate health effects on insects near operating nuclear power plants. The main result is a highly significant twofold increase in morphological malformations on true bugs in the 5 km vicinity of three Swiss nuclear power stations. Due to its ecological design, however, this study cannot answer the question whether the effect is caused by radiation from nuclear power plants. The result is unexpected as the dose from routine emissions of NPS is considered much too low to produce notable adverse health effects on biota. Our findings should be confirmed by future studies that avoid the shortcomings of the present study.

Experimental Section

True bugs and some leafhoppers were collected in the environs of three Swiss nuclear power stations:

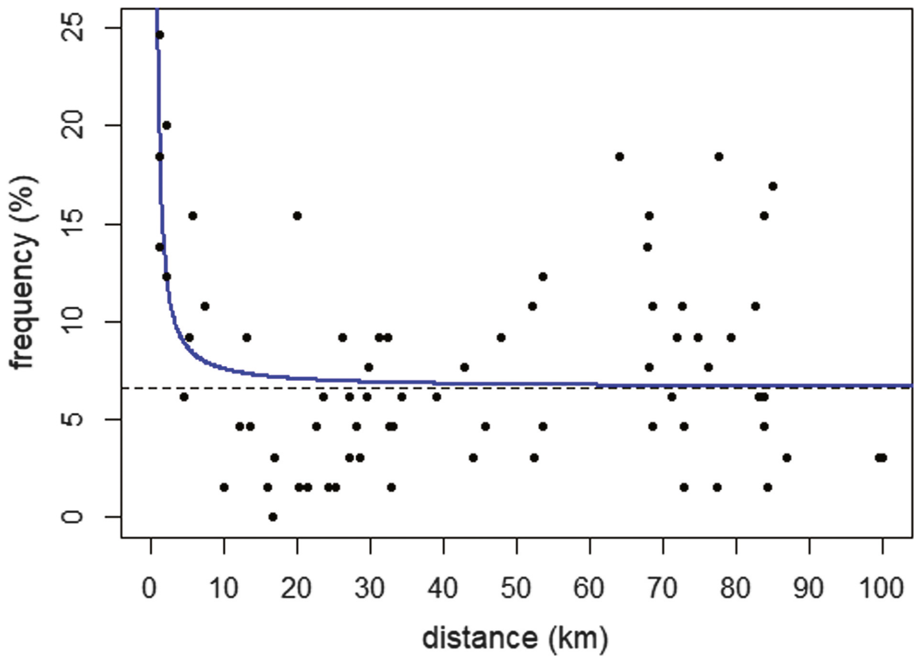

Figure 1. Frequency of abnormalities in true bugs in the environs of NPS Beznau and regression line. The broken line is the asymptotic frequency at large distances. The distance trend is highly significant ($P < 0.001$, *t*-test).

NPS Leibstadt, NPS Beznau with two reactors, and NPS Gösgen. The data were complemented by bugs collected in the biosphere Entlebuch, Canton Lucerne, 80 km away from NPS Leibstadt. A fixed number of 65 bugs were collected at each of 75 sites, and each site was geocoded. The collection sites and dates are available in the *Supporting Information Table S1*.

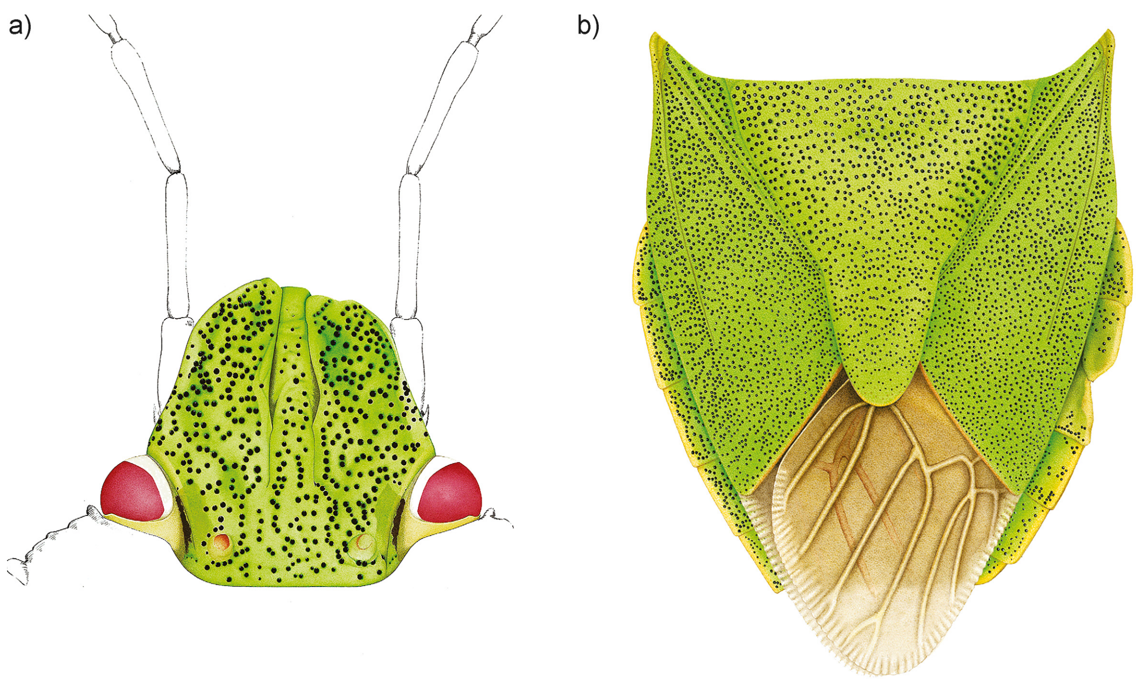

Figure 2. a) Head of stink bug, Pentatomidae from Hüttlenen, Canton Lucerne, Switzerland, which shows an abnormality on the right side. Watercolor, Zurich 2008. © *Cornelia Hesse-Honegger*. b) Scutellum, abdomen, and wings of stink bug, Pentatomidae, found close to Paul Scherrer Institute, Canton Aargau, Switzerland. The right side of the abdomen is abnormal. It shows a 'melted' shape. Watercolor, Zürich 2000, © *Cornelia Hesse-Honegger*.

The largest nuclear power plant is Leibstadt (KKL) at the river Rhine, a boiling water reactor (1275 MWel) which started operation in 1984. The two reactors at Beznau (KKB, 365 MWel each, 1969 and 1971) and the NPP Gösgen (KKG, 1060 MWel, 1979) are pressurized water reactors. The only other boiling water reactor is NPS Mühleberg (KKM, 373 MWel, 1971). However, no bugs were collected there, so this site is not included in this study. A nuclear research facility (Paul Scherrer Institute, PSI) is situated only about 1.5 km away from NPS Beznau, well within the 5 km range. Possible effects of PSI on abnormalities can therefore not be determined independently from NPS Beznau.

In the early 1990s, Hesse-Honegger differentiated between 'All Disturbances' and 'Morphological Disturbances', whereas 'All Disturbances' encompass dark patches, bent wings, little holes, white coating, and change in pigmentation (*Figure S1*). 'Morphological Disturbances' are more severe abnormalities. They

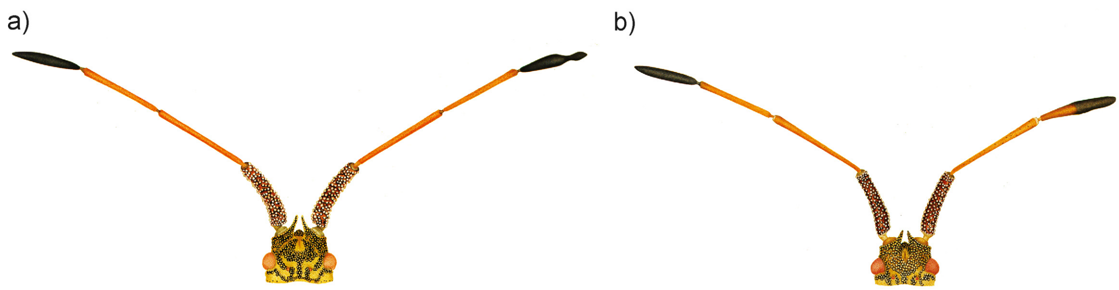

Figure 3. Head of squash bug, Coreidae, close to Paul Scherrer Institute, Canton Aargau, Switzerland. a) The point of the right antenna is short and disturbed. b) In the right antenna, a section is missing. Watercolor, Zurich, 2000, © *Cornelia Hesse-Honegger*.

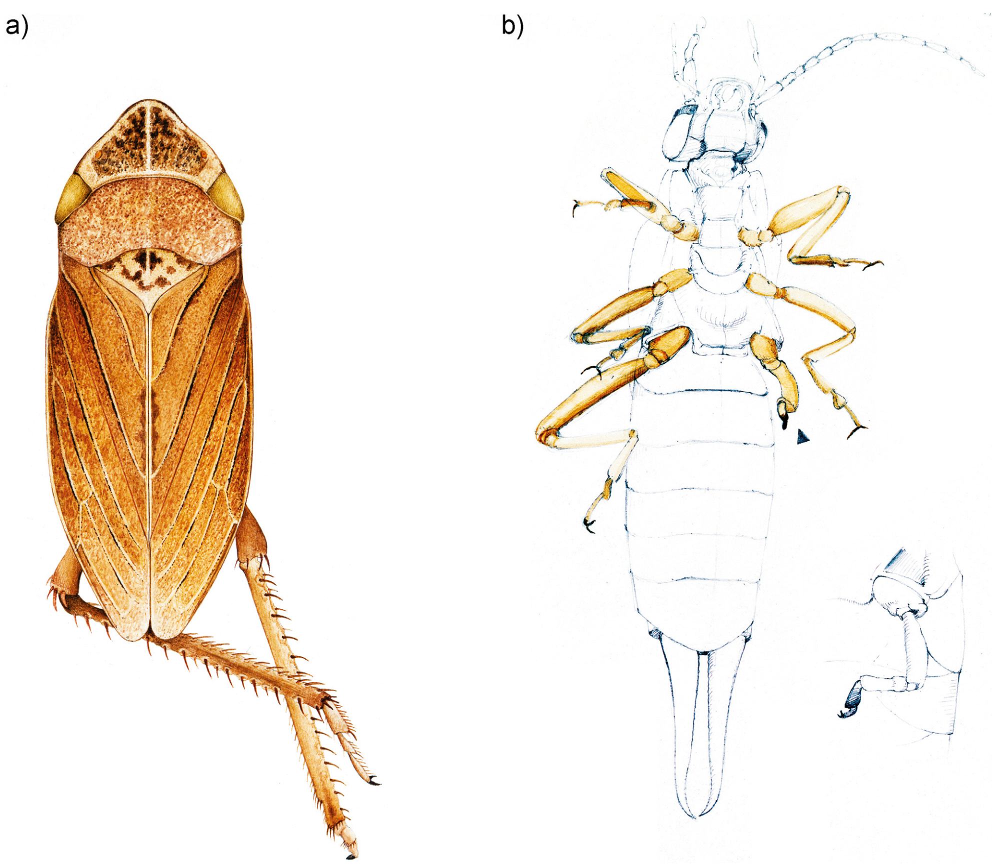

Figure 4. a) Leafhopper, Auchenorrhyncha from Würenlingen, Canton Aargau, Switzerland. The right hind foot is disturbed but with a tarsal claw. b) Ventral side of an earwig, Dermaptera from Reuenthal, Canton Aargau, Switzerland. The hind leg on the right side is short with a tarsal claw. Color sketch, Zürich 1988, © *Cornelia Hesse-Honegger*.

encompass changes in form, mostly asymmetries (*Figure 2,a*, *Figures S2* and *S5*), 'melted' chitin (*Figure 2,b*), fusion or shifting of adjacent abdominal tergites and sternites (*Figures S6* and *S7*), disturbed ommatidia and distribution of eye pigment on head (*Figure S3*), lack of 'point pits' (*Figure S4*), deformation in antennae or lack of sections (*Figure 3,a* and *b*), deformed leg or feet (*Figure 4*), wings in uneven length, and disturbance of patterns (*Figure S8*). For this study, only morphological disturbances were included.

Together with aphids (*Sternorrhyncha*), true bugs (*Heteroptera*) and leafhoppers (*Auchenorrhyncha*) belong to the phylum *Arthropoda*, class *Insecta*, order *Hemiptera*. Their piercing-sucking mouth piece extends a cannula by which they suck liquid out of plants. True bug larvae do not pupate and suck liquid of their host plants from the very first moment after hatching, when their size is < 1 mm. True bugs do not fly large distances, which is advantageous for field studies. A population can be observed over multiple generations in the same area. True bugs are widely distributed over the planet. Their families can be found on all continents.

True bugs were collected in plastic cups covered by paper tissue and closed by a rubber band. After administering an anesthesia (ethyl acetate), the bugs were inspected with a Leica MZ16 binocular microscope. Microscopic inspection comprised the outside shape of the insect, ventral, and dorsal. Inspection had to be finished within 12 h after collection, before stiffening and death of the insects.

Statistical Methods

First, a categorical analysis was carried out which compares the abnormality rates in samples < 5 km from the next NPS to the rates in samples more than 5 km away. The cut point of 5 km for the categorical analysis was chosen *a priori* because, in the KiKK (Kinderkrebs in der Umgebung von Kernkraftwerken) study, a significant increase of leukemia in young children was only found in the 5 km zone of German nuclear power stations.[6] A logistic regression was applied with a dummy variable *study* that denotes samples with distance $r < 5$ km. Second, abnormality rates were analyzed as a function of distance from each NPP site. We conducted logistic regression with reciprocal distance as a proxy for radiation exposure which was also used to model the distance dependency in the KiKK study. To account for overdispersion, i.e., greater dispersion than expected from a purely random distribution, an F test was applied for significance testing instead of a chi-square test. A two-sided $P < 0.05$ was considered statistically significant. The free software R was used for data analysis and plotting (http://www.r-project.org).

Supplementary Material

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201800099.

Author Contribution Statement

C. H.-H. collected the insects, determined the number of abnormalities in each sample, geocoded the sample sites, and wrote the experimental section. *A. K.* analyzed the data and wrote the rest of the article.

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