Faster Development Covaries with Higher DNA Damage in Grasshoppers (*Chorthippus albomarginatus*) from Chernobyl

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ABSTRACT

In Chernobyl, chronic exposure to radioactive contaminants has a variety of deleterious effects on exposed organisms, including genetic damage and mutation accumulation. However, the potential for such effects to be transmitted to the next generation is poorly understood. We captured lesser marsh grasshoppers (*Chorthippus albomarginatus*) in the Chernobyl Exclusion Zone from sites varying in levels of environmental radiation by more than three orders of magnitude. We then raised their offspring in a common garden experiment in order to assess the effects of parental exposure to radiation on offspring development and DNA damage. Offspring that reached maturity at a younger age had higher levels of DNA damage. Contrary to our hypothesis, parental exposure to radioactive contamination did not affect DNA damage in their offspring possibly because of intervening adaptation or parental compensatory mechanisms. Our results suggest a trade-off between developmental rate and resistance to DNA damage, whereby offspring developing at faster rates do so at the cost of damaging their DNA. This result is consistent with and extends findings in other species, suggesting that faster growth rates cause increased oxidative damage and stress. We propose that growth rates are subject to stabilizing selection balancing the benefits of fast development and the competing need of buffering its damaging effects to macromolecules and tissues.

Keywords: Chernobyl, DNA damage, fluctuating asymmetry, life history, oxidative stress, radiation.

Introduction

Natural habitats are frequently exposed to anthropogenic contaminants that pose challenges to their occupants. A number of studies have established that there is often a direct cost to organisms that live under chronic exposure to an environmental stressor, such as ionizing radiation (Zainullin et al. 1992; Møller and Mousseau 2006; Einor et al. 2016). Laboratory and field studies have also established that there are additional consequences for the offspring of exposed parents, including increased mutation rates (Carls and Schiestl 1999; Barber and Dubrova 2006; Natarajan 2006), increased birth abnormalities (Nomura 2006), as well as epigenetic modifications. Parents exposed to contaminants might be forced to reduce the amount and quality of resources that they contribute to their developing offspring (Mousseau and Fox 1998*a*; Mousseau et al. 2009). Epigenetic studies have shown that parental exposure to stressors can affect phenotypic expression and health in their offspring (Jirtle and Skinner 2007; Skinner et al. 2010; Perera and Herbstman 2011).

The meltdown of reactor 4 of the Chernobyl nuclear power plant in 1986 released massive quantities of radionuclides into the surrounding environment that persist today because of their long half-lives (Shestopalov 1996). Since the surrounding Chernobyl Exclusion Zone has been evacuated because of the high radioactive contamination, it provides an important site for studies of natural populations under varying levels of exposure to environmental radiation. Although the residual radionuclides amount to a relatively low dose rate, the cumulative dose over time is likely to lead to oxidative stress in exposed organisms (Bonisoli-Alquati et al. 2010*a*; Einor et al. 2016), DNA damage and an accumulation of unrepaired mutations (Bonisoli-Alquati et al. 2010*b*; Møller et al. 2010; Møller and Mousseau 2015), and epigenetic effects possibly magnifying the effects of changes in DNA sequence (Kovalchuk and Baulch 2008; Ilnytskyy and Kovalchuk 2011).

Laboratory studies on the effects of parental radiation exposure have demonstrated a variety of biological effects, including chromosomal inversions (Sykes et al. 2006; Zeng et al. 2006), mitotic recombination in fetal cells (Liang et al. 2007), point mutations (Liang et al. 2007; Schilling-Tóth et al. 2011), increased DNA strand breaks in sperm (Schindewolf et al. 2000; Dubrova 2005; Ozturk

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and Demir 2011), tumors that were inherited by the offspring of irradiated mice (Nomura 1982), and increased germline mutations that persisted into the F_1 and F_2 generations (Nomura 1990, 2006; Barber et al. 2002). Multigenerational studies of the model organisms *Daphnia magna* and *Caenorhabditis elegans* have shown both adaptation (Dutilleul et al. 2014) and sensitization (Alonzo et al. 2008; Parisot et al. 2015) as exposure to waterborne radionuclides or external cesium 137 gamma radiation continued into the F_1 and F_2 generation. To make predictions of adaptation dynamics and ecological risk more complicated, pollution adaptation was found to entail adaptation costs when the organisms were returned to an uncontaminated environment (Dutilleul et al. 2017).

Multigenerational studies on natural populations in such environments are rare, and their inherited effects are poorly understood. Notably, studies of the pale blue grass butterfly *Zizeeria maha* in the area contaminated by the accident at the Fukushima-Daiichi nuclear power plant have discovered morphological abnormalities that persist in both the F_1 and F_2 generations reared under controlled lab conditions (Hiyama et al. 2012; Taira et al. 2014). Further, studies of human populations exposed to the aftermath of the Chernobyl disaster have demonstrated increased microsatellite mutation rates (Dubrova et al. 1996; Weinberg et al. 2001; Furitsu et al. 2005) and increased cellular abnormalities and chromosomal breaks (Aghajanyan et al. 2011) in children born to parents exposed to radiation after the Chernobyl accident.

Here, we hypothesized that grasshoppers born from parents collected from highly contaminated sites would display higher levels of DNA damage. Parents in areas with higher levels of radioactive contamination must use energy and antioxidant resources for surviving under such conditions and are thus likely to have reduced resources and antioxidants available for provisioning their eggs (Møller et al. 2005, 2008). An increase in DNA damage in the offspring of parents exposed to radioactive contamination could also arise if higher mutation rates and/or methylation changes impaired the offspring's DNA repair machinery. We also tested the hypothesis that grasshoppers with higher DNA damage have higher levels of fluctuating asymmetry, which constitutes a phenotypic marker of the inability to compensate for developmental errors (Møller and Swaddle 1997; Beasley et al. 2013). We expected fluctuating asymmetry to increase at higher levels of DNA damage as an overall indicator of susceptibility to the negative effects of environmental stress.

Material and Methods

Study Species, Field Procedures, and Lab Rearing Conditions

The brown form of the lesser marsh grasshopper (*Chorthippus albomarginatus*) is a medium-sized univoltine orthopteran. Its extensive range throughout Europe spans the southern half of Scandinavia and Finland and ranges south into Spain and Italy and east into Ukraine (Vedenina and Helversen 2003; Walters et al. 2006). The southern margins of *C. albormarginatus* habitat are constrained by warmer temperatures and arid climates (Langmaack and Schrader 1997). The species can be found throughout the Chernobyl Exclusion Zone and surrounding areas in significant numbers. The grasshoppers tend to aggregate in areas with an open canopy, remaining on the ground during the day and climbing into the vegetation at night. About a week after adult emergence, females oviposit multiple egg pods into soft, loose soil. Following an obligatory overwintering diapause, nymphs typically emerge between May and June (Walters et al. 2006).

Adult *C. albomarginatus* were collected using a standard insect sweep net from six different locations in the Chernobyl area during September 20–26, 2009. All sites were grassy fields with loam soil, surrounded by forests. Environmental radiation levels ranged from 0.03 to 50.06 microsieverts per hour (μSv/h; fig. 1). Environmental radiation levels at the site of collection were measured using a handheld dosimeter (Inspector, SE International, Summertown, TN) placed about 1 cm above the ground to simulate the dose received by grasshoppers. Although we did not estimate the dose received, we expect it to be strongly positively correlated with background radiation levels measured at the collection sites because this species has very short dispersal distances relative to the scale of variation in background radiation (Shestopalov 1996). We recently validated this assumption in birds (A. Bonisoli-Alquati, T. Mappes, G. Milinevsky, A. P. Møller, T. A. Mousseau, and D. Tedeschi, unpublished data), a group far more mobile than our study species. We report ambient radiation levels in microsieverts per hour. Summary statistics for each population by collection site are provided in table 1.

Following collection from the field, adults were brought to a laboratory with low ambient radiation levels (0.06–0.12 μSv/h) and left to acclimate for 3 d before mating. Following acclimation, females were paired with a male from the same source population to allow for egg fertilization in any females who had not already mated before capture. The females were placed in Styrofoam cages (height, ca. 12 cm; diameter, ca. 8 cm) with sterilized sand for oviposition and fresh lettuce and a carrot for sustenance. Plastic cups (height, ca. 10 cm; diameter, ca. 8 cm) were used to seal the cages. The sand was sifted once each day after mating in order to extract egg pods if present. Following oviposition, the number of eggs within egg pods were counted for each female and placed in clean vials containing moistened sterilized vermiculite in order to provide sufficient moisture for development and inhibit microbial and fungal growth.

Following their arrival at the lab, the egg pods were maintained at 4°C for 3 mo to break diapause and then shifted to 24°C to initiate postdiapause development and hatching. Egg pods were monitored twice daily, and newly emerged nymphs were transferred to $9 \times 9 \times 8$-cm^3 plastic cages (mean hatching success = 88.07% [15.78 SD], $n = 1,601$). Cages housed only siblings, and density averaged nine nymphs per cage (4.0 SD) for the duration of nymphal development. Cages were changed to maintain a clean environment, and fresh food was provided twice weekly. Nymphs were fed a diet of organic lettuce, carrots, and wheat germ and provided with a strip of paper towel for cover. Carbon dioxide (CO_2) was used to anesthetize nymphs before transferring them to clean cages to minimize handling stress. This exposure was brief, and any stress associated with anoxia related to CO_2 was experienced to an equal extent by all grasshoppers included in this study, and so it should not account for any relative differences among

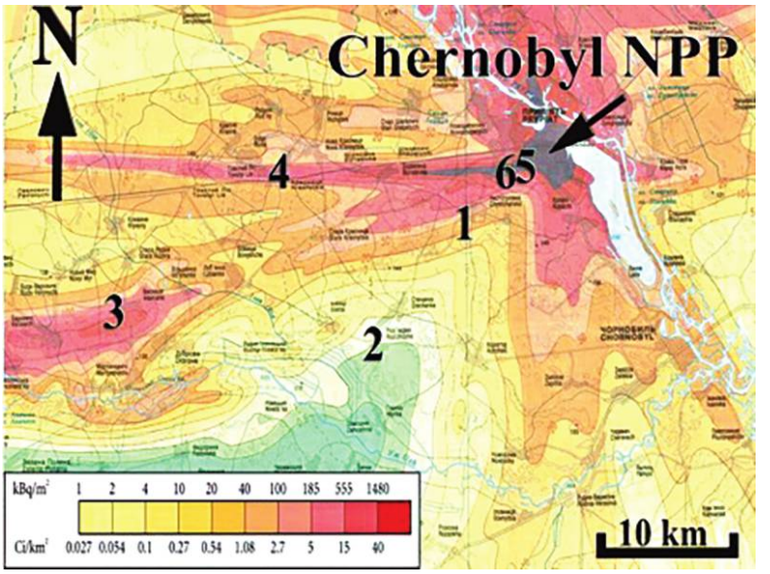

Figure 1. Map of study sites. Locations of study sites where the parent grasshoppers of the individuals included in this study were collected are indicated on a map of radioactive contamination by cesium 137 (1, Chystohalivka; 2, Rozizhdzhe; 3, Vesniane; 4, Krasnytsia; 5, Red Forest 1; 6, Red Forest 2). Adapted from Shestopalov (1996). Chernobyl NPP, Chernobyl nuclear power plant.

groups (Colinet and Renault 2012). Some nymphs died from natural causes before reaching maturity and hemolymph extraction (484 of 968 nymphs died). These nymphs were included only in population-level analyses of mortality. Nymph cages were maintained in an incubator set at a constant temperature and photoperiod (28°C, 15L:9D) to complete development to the adult (sixth) instar and were monitored daily for the presence of adults. Adults were removed from nymphal feeding cages, weighed with a Sartorius Research electronic balance (model R160P; to 0.001 g) within 12 h after emergence, and then placed in individual cages with organic lettuce and a carrot slice. To maintain a hygienic environment, adults were transferred to clean cages and provided fresh food twice weekly. Following death, adults were removed from cages and stored in 95% ethanol for later analysis.

Offspring Phenotypic Measurements and Hemolymph Collection

We collected three life-history and morphological variables in the offspring: time to maturity (the number of days from hatching to adult emergence, indicating sexual maturity), body mass at sexual maturity (body mass hereafter), and pronotum length as a measure of adult body size (accuracy 0.1 mm). Within each family, we calculated within-family survival rates as the ratio between the number of surviving nymphs and the total number of hatched nymphs.

Seven days after adult emergence of the first individual in each family, we collected hemolymph from each grasshopper. Adult grasshoppers were placed on ice for 5 min to anesthetize them before hemolymph extraction. We carefully inserted a microsyringe (Hamilton Microsyringe, 50 μL) into the ventral side of the abdomen and extracted approximately 5 μL of hemolymph from each individual and placed into a microcentrifuge tube containing 50 μL of $\times 1$ phosphate-buffered saline (PBS) and maintained on ice. The syringe was flushed with 95% ethanol between each extraction.

Comet Assay

The alkaline (pH $>$ 13) comet assay (single-cell gel electrophoresis) is a commonly used method to estimate DNA breakage in vivo resulting from exposure to environmental toxins and stressors (Meehan 2004; Bonisoli-Alquati et al. 2010*b*; Almeida et al. 2011; Monteiro et al. 2011). The alkaline version of the assay can detect a variety of classes of DNA damage, including single-strand breaks, alkali-labile sites, and DNA crosslinking (Tice et al. 2000). This technique has been successfully utilized on insect populations to test responses to electron beam sterilization for commercial food stocks (Imamura et al. 2004; Todoriki et al. 2006) and to assess in vivo DNA damage in *Drosophila* larvae from exposure to a chemical toxin (Carmona et al. 2011). The comet assay is sensitive enough to detect even very small differences in DNA damage (Gotoh and Kikuchi 2001), and its use with hemocytes has been previously successfully validated in insects (Carmona et al. 2011) as well as other invertebrates (Rigonato et al. 2005).

We performed the comet assay using the protocol described by Singh et al. (1988), with minor modifications. All steps were performed under incandescent light to prevent additional DNA damage. Single-frosted slides (VWR, Radnor, PA) were prepared in advance by dipping the slides in 1.5% normal melting point agarose (BioRad, Hercules, CA) twice; the backs of the slides were then wiped clean, and the slides were allowed to dry for at least 24 h before use for the comet assay. Approximately 5 μL of hemolymph

Table 1: Summary data for samples analyzed for each site

	Chystohalivka	Rozizhdzhe	Vesniane	Krasnytsia	Red Forest 1	Red Forest 2
Radiation (μSv/h)	.03	.10	5.40	10.72	35.31	50.06
No. individuals	10	36	51	27	45	12
No. families	6	17	23	12	18	4
Mean DNA damage ± SD	37.98 ± 22.19	33.39 ± 9.94	39.60 ± 14.86	38.37 ± 12.07	33.73 ± 8.39	43.94 ± 18.04
SE	7.02	1.66	2.08	2.32	1.26	5.2
80th DNA damage ± SD	46.50 ± 22.95	42.83 ± 10.56	49.96 ± 16.51	50.36 ± 15.72	42.04 ± 9.93	55.36 ± 21.32
SE	7.25	1.76	2.31	3.02	1.5	6.16
Days to maturity ± SD	5.9 ± 5.8	62.7 ± 4.8	59.2 ± 8.4	57.2 ± 6.2	61.3 ± 4.0	57.6 ± 7.0
SE	1.85	.8	1.17	1.2	.61	2.02
Survival rate ± SD	57.49 ± 24.78	41.17 ± 25.22	44.73 ± 20.82	51.99 ± 23.03	54.33 ± 23.39	68.21 ± 14.51
SE	10.11	6.12	4.34	6.65	5.51	7.26

Note. Averages per site are reported together with their associated standard deviations and standard errors. Radiation indicates environmental radiation levels to which the parental generation was exposed. Mean DNA damage (and 80th percentile DNA damage) was calculated for each individual grasshopper as the average (or 80th percentile) of the distribution of >80 cells. Survival rate was calculated in each family as percent survival of offspring within a family to adulthood.

in 50 μL of × 1 PBS was added to 450 μL of 1% low melting point agarose (Amresco, Solon, OH), and 100 μL of the agarose mixture was immediately layered onto the prepared slides and covered with a glass coverslip and then allowed to solidify for 5 min at 4°C. The coverslip was then removed and a second layer of 100 μL of low melting-point agarose was layered on top of the first and covered again with a coverslip, which was removed after 5 min. Two samples were placed on each slide, with a total of four replicates for each individual. The slides were allowed to incubate for 1 h at 4°C to allow the gel to fully solidify. The slides were then immersed in cold lysis buffer (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% Triton X-100 [pH 10], with the Triton X-100 added immediately before use) and kept for 1 h at 4°C. The slides were rinsed with cold ddH_2O and then immersed in alkali buffer (300 mM NaOH, 1 mM Na_2EDTA, pH $>$ 13) to allow the DNA to unwind for 1 h at 4°C. Electrophoresis was conducted using the alkali buffer for 30 min at 0.7 V/cm and 100 mA at 4°C. We rinsed the slides three times for 5 min each in a neutralization buffer (0.4 M Tris, pH 7.4) followed by 15 min in 70% ethanol. The slides were then placed in a darkened cupboard and allowed to dry overnight before storage in a dark slide box.

Slides were stained using a 1 : 10,000 dilution of SYBR Gold (Trevigen, Gaithersburg, MD), and images were captured using a Zeiss Axioskop D40 microscope at × 40 magnification equipped with an AxioCam HRM camera and utilizing the AxioVision software package (Carl Zeiss, Thornwood, NY). The images were analyzed using CometScore 1.5 (TriTek 2003). Each sample was run in replicate on two or more separate slides (two slides, $n = 129$; three slides, $n = 28$; four slides, $n = 15$). An approximately equal number of cells were scored from each slide. The main measure of DNA damage used here and derived from the assay (i.e., the proportion of DNA in the tail) was highly repeatable across slides in a one-way ANOVA with individual identity as the only factor ($F_{171, 230} = 6.53$, $r^2 = 0.829$, $P < 0.0001$). All samples were processed and scored blind. Mean cell damage and 80th percentile were calculated for each individual. Before analyses, mean DNA damage and 80th percentile DNA damage were log transformed to attain normality.

Wing Measurements

Wing measurements were estimated as previously described by Beasley et al (2012). Briefly, the forewings and hind wings were removed from the thorax, wet mounted on glass microscope slides with distilled water, and covered with a coverslip. Slides were allowed to dry overnight at room temperature (24°C) and then sealed using coverslips. Slides were then scanned at a resolution of 2,400 dpi.

All landmarks were located at wing-vein intersections and termination points (fig. 2) and were anatomically homologous

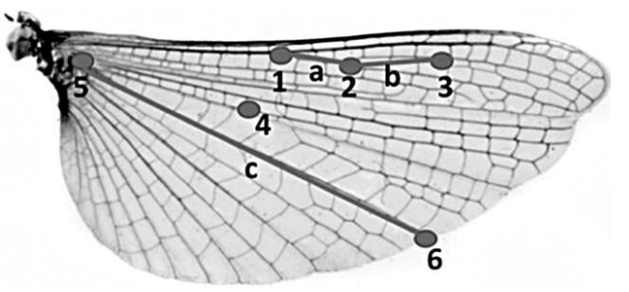

Figure 2. Landmarks for fluctuating asymmetry analysis. Hind wing of a lesser marsh grasshopper (*Chorthippus albomarginatus*) showing landmark positions on wing veins (numbers) and vein lengths (letters) selected for fluctuating asymmetry measurements with image analysis. Adapted from Beasley et al. (2012). A color version of this figure is available online.

among all individuals, thus fulfilling the criterion for type I landmarks (Bookstein 1991). Landmarks were digitized on the left and right hind wing using TpsDig2.16 software (Rohlf 2005). All wings within the sample were measured three times to allow assessment of digitizing error with a Procrustes ANOVA with wings as the side effect, digitizing as the error effect, and ID as the individual effect (Klingenberg and McIntyre 1998). A Procrustes ANOVA found the between-sides variation to far exceed the measurement error ($F_{72} = 12.09$, $r^2 = 0.97$, $P < 0.0001$) and scanning technique ($F_{72} = 17.20$, $r^2 = 0.99$, $P < 0.0001$). Measurement error due to mounting was very low for both the left wing ($F_{26} = 699.23$, $r^2 = 0.99$, $P < 0.0001$) and the right wing ($F_{26} = 245.86$, $r^2 = 0.99$, $P < 0.0001$), with repeatability in excess of 99.43% (Beasley et al. 2012). Furthermore, we confirmed the absence of directional asymmetry and antisymmetry (Beasley et al. 2012). Mean landmark coordinates calculated by averaging the three measurements per landmark for each individual were used in the analysis (fig. 2).

We used the software MorphoJ (Klingenberg 2011) to extract size information from the raw data, using a full Procrustes fit to control for wing orientation and position and a Procrustes ANOVA to calculate centroid sizes (Klingenberg and McIntyre 1998; Klingenberg et al. 2002). Centroid size is the square root of the sum of squared distances of a set of landmarks (Klingenberg and Monteiro 2005; Rohlf 2005). As an index of fluctuating asymmetry, we used the unsigned difference in centroid size between the right and the left wing.

Statistical Analyses

We ran the analyses mainly by relying on linear mixed models using JMP Pro 13 (SAS Institute, Cary, NC). In the analyses of DNA damage as a function of parental exposure to radiation and offspring life history, we a priori considered alternative models and compared their fit statistics. The most complete model included individual sex (as a fixed factor), radiation level, time to maturity, and time to maturity squared to account for potential nonlinear trends in the relationship (as covariates). In addition, in the full model we also tested all two-way interactions between factors and covariates. In all models, collection site and identity of the family of origin (nested within the site) were added as random effects. We compared a priori alternative models on the basis of their corrected Akaike information criterion (AICc) and Bayesian information criterion (BIC) fit statistics (appendix). Here, we present the results of the most informative models.

We also tested whether higher DNA damage was associated with higher fluctuating asymmetry, as indexed by asymmetry in wing centroid size. In these models, we included sex, parental exposure to radiation, time to maturity, time to maturity squared, and either mean DNA damage or 80th percentile DNA damage (as covariates). In order to test for sex-specific susceptibility, we also tested for the effects of all the two-way interactions between sex and the different covariates. We compared a priori alternative models on the basis of their AICc and BIC fit statistics (appendix).

We estimated the effects of parental radiation exposure, DNA damage, and wing asymmetry on mortality by analyzing within-family survival rate (hatching to adulthood) as a function of radiation exposure by means of a logistic regression using an events/trials syntax in JMP Pro 13. In these models, we assumed a binomial error distribution and used a logit link function. The response variable was the ratio of surviving nymphs (events; $N = 484$) to the overall number of hatched nymphs (trials; $N = 968$). Thus, estimates of within-family mortality rates were based on all hatched individuals and not strictly on those for which we had obtained information on DNA damage.

In order to further test whether differences in maturation time were in fact due to differences in parental radiation exposure, we classified maturation times in three categories, using the 33rd and 66th percentiles of the distribution as cutoffs. We then tested for an overall difference in parental radiation exposure in the three resulting categories. This analysis allowed us to test whether different growth strategies (i.e., fast development/high DNA damage vs. slow development/low DNA damage) were emerging along the cline of radiation exposure because of either plasticity or adaptation to radiation exposure. The data described in this article are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9v8c4.

Results

DNA Damage and Life History

We obtained information on DNA damage for 178 individuals for which we could score ≥80 cells (mean number of cells = 99.14 [3.20 SD]; range: 80–100). Mean DNA damage in our sample averaged 36.83% (0.98 SE), while 80th percentile DNA damage averaged 46.69% (1.11 SE). Time to maturity was on average 60 d (0.5 SE). The best-fit models for mean and 80th percentile DNA damage included time to reach maturity and time to reach maturity squared (for fit statistics of the best model and the alternative models, see table A1). DNA damage increased as the time to reach maturity shortened (fig. 3; mean DNA damage: slope [SE] = −10.64 [1.54], $F_{1, 174.8} = 28.73$, $P < 0.0001$; 80th percentile DNA damage: slope [SE] = −10.28 [1.64], $F_{1, 103.6} = 39.38$, $P < 0.0001$). In addition, the quadratic term was also significant, indicating that the relationship between time to maturity and DNA damage was reversed for extremely long maturation times (fig. 3; mean DNA damage: slope [SE] = 8.06×10^{-2} [1.70×10^{-2}], $F_{1, 174.8} = 22.46$, $P < 0.0001$; 80th percentile DNA damage: slope [SE] = 7.62×10^{-2} [1.42×10^{-2}], $F_{1, 92.3} = 28.96$, $P < 0.0001$). To further investigate the relationship between DNA damage and time to maturity, we fitted a break point regression to the data. The analyses indicated the existence of one break point in the data at a duration of 58.12 d (95% confidence interval [CI], 56.39–59.86; $P < 0.0001$). At the break point, the slope significantly changed from −2.50 (0.22 SE; 95% CI, −2.93 to −2.07) to −0.0912 (0.22 SE; 95% CI, −0.5188 to 0.3364).

In the analysis of mean DNA damage, the effects of collection site and family of origin (as random effects) were significant (Wald $P < 0.0001$). In the analysis of 80th percentile DNA damage, the effect of family of origin (but not the one of the collection site) was significant (Wald $P < 0.0001$). Contrary to our prediction, the

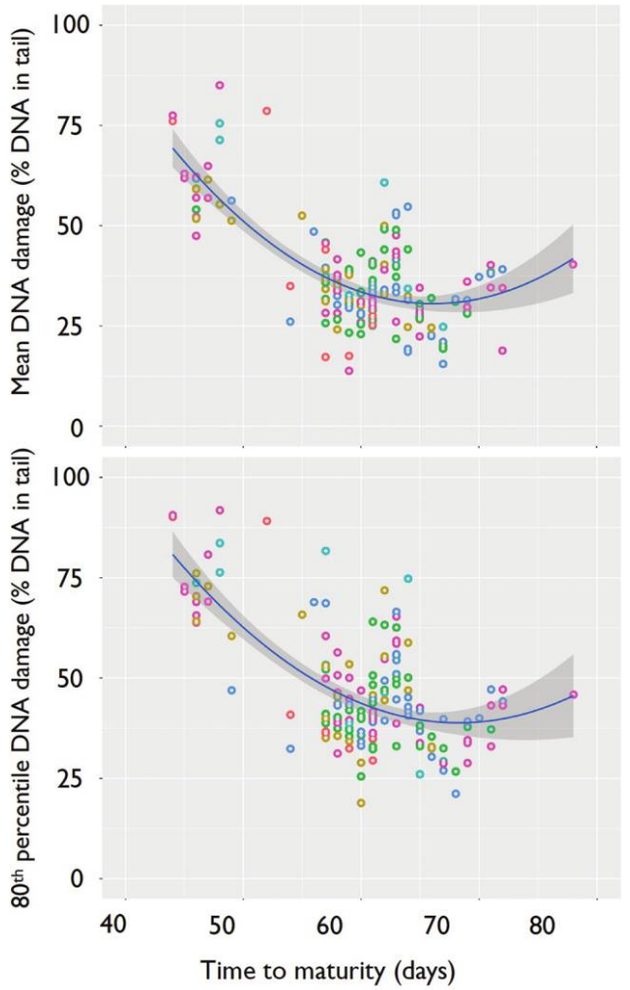

Figure 3. DNA damage in grasshopper hemolymph cells. Mean DNA damage (i.e., mean percent DNA in tail of comets calculated for each individual; *top*) and 80th percentile DNA damage in hemolymph cells as a function of time to reach maturity (in days; *bottom*). Points are color coded according to the site of origin (red, Chystohalivka; blue, Rozizhdzhe; pink, Vesniane; yellow, Krasnytsia; green, Red Forest 1; light blue, Red Forest 2). Second-degree polynomial regression fit lines are shown to indicate the significance of time to maturity squared.

relationship between parental radiation exposure and DNA damage was not significant in any of the assessed models (fig. 4).

Fluctuating Asymmetry, DNA Damage, and Survival

We analyzed variation in wing fluctuating asymmetry (as indexed by asymmetry in centroid size) in a series of models that included parental radiation exposure, time to maturity, time to maturity squared, and either mean or 80th percentile DNA damage. The best-fit model included only parental radiation exposure (table A2). Higher levels of parental radiation exposure correlated with higher wing fluctuating asymmetry, although the effect was marginally nonsignificant (fig. 5; slope [SE] $= 3.8 \times 10^{-5}$ [1.9×10^{-5}], $F_{1, 122} = 3.84$, $P = 0.052$). The effect of DNA damage was not significant (slope [SE] $= -4.7 \times 10^{-5}$ [8.6×10^{-5}], $F_{1, 122} = 0.30$, $P = 0.582$). The effects of collection site and family of origin (as random effects) were both significant (Wald $P < 0.0001$).

To test the hypothesis that radiation, higher genetic damage, and higher fluctuating asymmetry would increase mortality, we analyzed survival rate (hatching to adulthood) within each family as a function of within-family average DNA damage (i.e., the average of within-family mean or 80th percentile DNA damage), asymmetry in centroid size, and parental radiation exposure. Neither mean DNA damage nor 80th percentile DNA damage significantly predicted survival rate (table 2; fig. 6*a*). Parental radiation exposure positively predicted survival rate (table 2; fig. 6*b*). Wing asymmetry tended to increase with survival rate, although the effect was marginally nonsignificant (table 2; fig. 6*c*).

Studies on the offspring of insects exposed to radioactive contamination have shown longer maturation times (Taira et al. 2014). Thus, to test the hypothesis that different developmental times were also a result of parental radiation exposure, we divided the distribution of developmental times into three intervals defined by the 33rd and the 66th percentiles of the distribution and tested for a difference in parental radiation exposure. However, parental radiation exposure did not differ significantly among the three groups (one-way ANOVA; $F_{2, 178} = 1.37$, $P = 0.256$).

Discussion

The main findings of this study were that (1) DNA damage in individual grasshopper offspring was negatively correlated with development times and (2) was not significantly related to parental exposure to ionizing radiation, and (3) offspring survival was higher in families from sites that were highly contaminated by

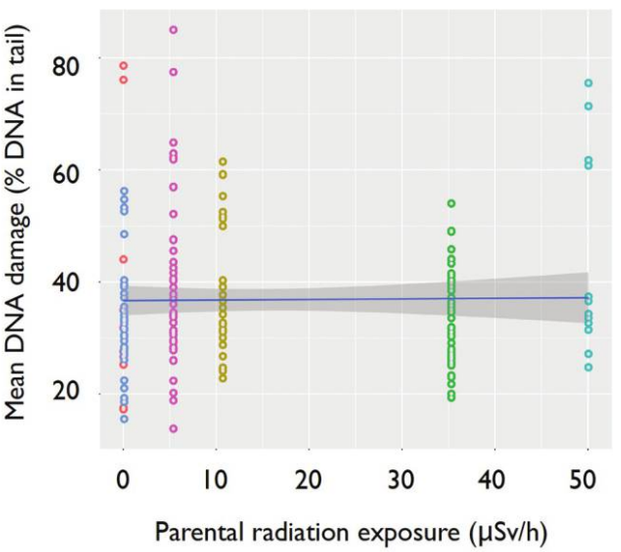

Figure 4. DNA damage in grasshopper hemolymph cells as a function of parental radiation exposure. Points are color coded according to their site of origin (red, Chystohalivka; blue, Rozizhdzhe; pink, Vesniane; yellow, Krasnytsia; green, Red Forest 1; light blue, Red Forest 2).

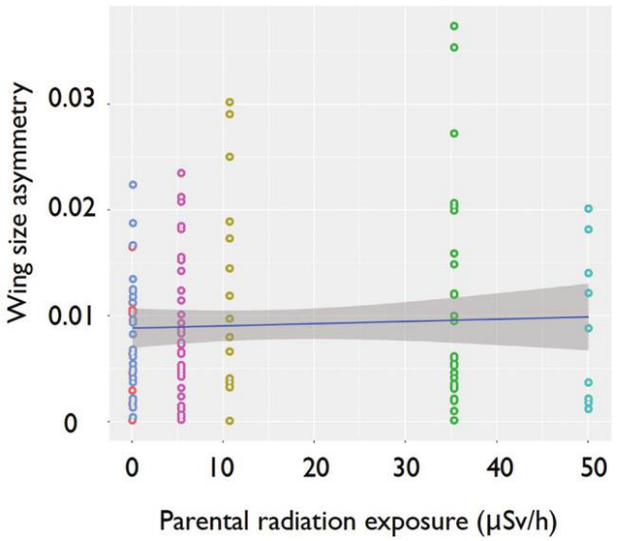

Figure 5. Asymmetry in centroid size as a function of parental radiation exposure. Points are color coded according to the site of origin (red, Chystohalivka; blue, Rozizhdzhe; pink, Vesniane; yellow, Krasnytsia; green, Red Forest 1; light blue, Red Forest 2).

ionizing radiation. The relationship between shorter developmental times and higher DNA damage suggests the existence of a trade-off between growth rate and genetic integrity. While rapid growth to a larger size is likely to provide a more immediate fitness advantage, the associated higher production of reactive oxygen species leaves an individual exposed to genetic damage that may have negative consequences later in life and may even be transmitted to offspring through damaged gametic DNA or a predisposition toward reduced ability to repair damage (Ward 1988; Weinberg et al. 2001; Dizdaroglu et al. 2002; Monaghan et al. 2009; Aghajanyan et al. 2011).

Compensatory growth is unlikely to be a factor in our results because all grasshoppers were supplied with ample food and time for growth, and no outside stressors were introduced to repress early growth in a way that might lead to compensatory growth and the accompanying increase in oxidative stress (Stoks et al. 2006; DeBlock and Stoks 2008; Dmitriew 2011; Stoks and DeBlock 2011). Rather, all organisms received equal resources, time, space, and other environmental conditions. Consistent with this but contrary to our predictions, parental radiation exposure did not account for variation in DNA damage in any analysis. Parental radiation exposure also did not correlate with the relationship between developmental times and DNA damage. We had predicted higher genetic damage in the offspring of parents from more highly contaminated areas on the basis that parents exposed to ionizing radiation contributed fewer antioxidants to their eggs because of the trade-off that they experience in the use of antioxidants. Our results rather indicate that the observed differences in genetic damage resulted from individual differences, and therefore the oxidative cost of rapid growth is important regardless of early growth conditions. They also indicate that historical exposure to ionizing radiation did not select for higher resistance of DNA to oxidative damage during development by allowing faster development to happen without consequences for genetic integrity. Because of the correlational nature of this analysis, it is not possible to confirm that higher DNA damage in faster-developing grasshoppers was due to increased production of reactive oxygen species. It is also difficult to interpret the differences among sites and among families that we disclosed in the analyses of DNA damage. While our collection sites were ecologically similar, we did not collect information related to ecological factors that could conceivably influence parental reproductive strategies, including provisioning of the eggs. Such factors include both physical characteristics (e.g., soil pH, temperature, and precipitation) as well as biological characteristics (e.g., food abundance, density of predators, competitors, or parasites). The relevance of these factors in determining DNA damage in developing grasshoppers remains to be tested. Future experimental studies with antioxidant supplementation to growing grasshoppers and/or enzymatic modifications of the comet assay allowing detection of oxidative damage to DNA might explicitly test whether variation in DNA damage in our sample was due to variation in oxidative insult to the hemocytes. Further analyses of the mechanism behind the observed genetic damage could also provide insight into how organisms provision their offspring in order to prepare them for the environment in which they will hatch (Mousseau and Fox 1998*b*). The

Table 2: Logistic regression models

	Wald χ^2	P	Slope (SE)
Mean DNA damage:			
Radiation	8.08	.004	1.1×10^{-2} (4.0×10^{-3})
Mean DNA damage	1.24	.265	-5.7×10^{-3} (5.1×10^{-3})
Wing asymmetry	3.48	.062	29.68 (15.90)
80th percentile DNA damage:			
Radiation	8.10	.004	1.2×10^{-2} (4.0×10^{-3})
80th percentile DNA damage	3.02	.082	-8.3×10^{-3} (4.8×10^{-3})
Wing asymmetry	2.65	.103	27.27 (16.12)

Note. Logistic regression models of within-family survival rate of grasshoppers (=number of nymphs surviving to adulthood over the total number of hatched nymphs) as a function of parental exposure to radiation, average within-family unsigned wing asymmetry, and within-family average mean DNA damage or 80th percentile DNA damage (for more details, see "Statistical Analyses").

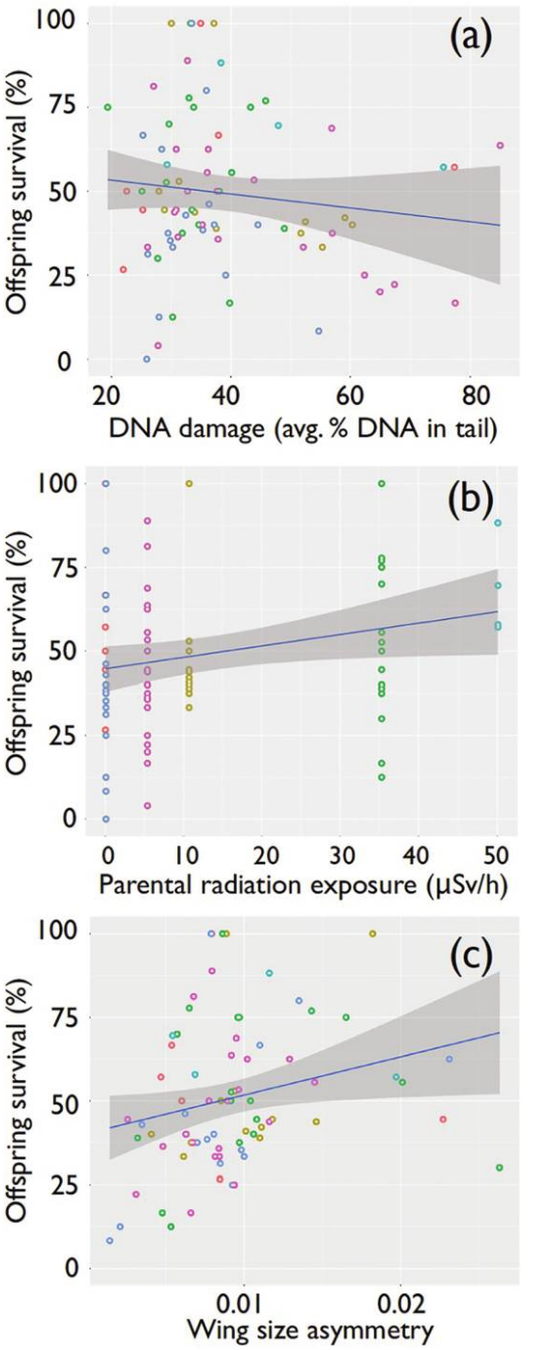

Figure 6. Family survival rate analysis. Within-family survival rate (from hatching to adulthood) of offspring grasshoppers (=number of surviving nymphs/total number of nymphs) as a function of average of mean DNA damage within the family (*a*), parental exposure to radiation (*b*), and within-family average wing asymmetry (*c*). Wing asymmetry was calculated as the absolute difference between centroid size of the right and the left wing (for more details, see "Material and Methods"). Points are color coded according to the site of origin (red, Chystohalivka; blue, Rozizhdzhe; pink, Vesniane; yellow, Krasnytsia; green, Red Forest 1; light blue, Red Forest 2). Lines represent simple linear regression lines. Equations for the reported linear regression lines are $y = 57.4 - 0.2x$ (*a*), $y = 44.7 + 0.3x$ (*b*), and $y = 40.3 + 1{,}144.6x$ (*c*).

seasonal boundaries of these grasshoppers' reproductive stage and relatively long development time (Vedenina and Helversen 2003; Walters et al. 2006) suggest that adult longevity is primarily important to the extent that an individual survives through the end of the temperate zone mating season to increase the probability of producing higher numbers of offspring. Therefore, establishing more immediate possible benefits (i.e., reliable sexual signaling, higher fecundity) for gaining reduced DNA damage at a cost of delayed adult emergence would provide a better explanation for the negative correlation between development rates and genetic damage. Experimentally exposing the offspring of wild-caught grasshoppers from these source populations to different radiation doses might elucidate whether the reduced genetic damage would become more immediately important under conditions of environmental stress.

In this study, the grasshoppers in the F_1 generation were not directly exposed to ionizing radiation and were raised in a uniform, well-provisioned environment. In spite of the standardization of growing conditions, the offspring of parents from contaminated sites were more likely to survive to maturity than the offspring of parents from clean areas. This difference was not explained by differences in maturation times between contaminated and clean areas or by differences in clutch size or mass. At present, any explanation is thus deemed to be highly speculative. One possibility is that grasshopper mothers in contaminated areas transferred more nutrients or larger amounts of other compounds (e.g., antioxidants) in order to compensate for the suboptimal conditions that their offspring would be growing in. Such provisioning would translate into higher survival under the benign lab conditions where their offspring will grow. It could also explain why we did not detect the predicted increase in DNA damage in the offspring of exposed parents. To test for a potential role of maternal effects via the egg in promoting offspring survival, future studies will need to control for or manipulate egg quality in grasshopper offspring exposed to contrasting conditions of environmental contamination.

It is important to note that we selected this insect species for our study precisely because it is found in significant population densities throughout the Chernobyl Exclusion Zone, even in areas where many species of birds, insects, and mammals show reduced population densities, presumably because of their sensitivity to the effects of ionizing radiation (Møller and Mousseau 2007, 2009, 2013; Møller et al. 2012). This indicates that these grasshoppers might already be partially adapted to their environment, which is consistent with a study on birds in the Chernobyl Exclusion Zone (Galván et al. 2014) and supported by the observed positive relationship between survival rates and radiation. Experimentally exposing species that are no longer found in highly contaminated areas to varying levels of radiation in a laboratory could elucidate species-level differences in response to this type of environmental stressors.

Future characterization of the particular radionuclides that contributed to radiation dose in the exposed parental generation will be important for understanding the relationships documented here. A complex mixture of radionuclides is found in the contaminated sites in Chernobyl where we collected the parents, including cesium 137, strontium 90, americium 241, and several

isotopes of plutonium. While gamma radiation is the most typical contributor to radiation dose in Chernobyl, highly energetic alpha particles from actinides and beta particles from most radionuclides are also present. Even though they are less common, actinides have slower turnover rates within the body and therefore can damage biological macromolecules for longer once assimilated. In our study, we did not quantify the relative contribution of the different radionuclides to body burden of radiation in the exposed parents, nor did we quantify their respective abundance in the environment. In fact, we measured environmental levels of gamma radiation and used them as proxies for a relative comparison between the offspring of exposed and unexposed parents. Such an approach has previously been shown to accurately reconstruct radiation exposure in other taxa (Garnier-Laplace et al. 2015). Nonetheless, future studies might benefit from more precise dose estimates in order to understand the mechanisms mediating the effects documented here.

The use of the comet assay is relatively new to studies on insect populations and has primarily been applied to toxicological assessments on the efficacy of pesticides (Imamura et al. 2004; Todoriki et al. 2006). Although hemolymph has been widely attributed to insect immune function, these cells remain relatively poorly understood and a potentially useful candidate for nondestructive studies on insects, particularly given their responses to experimental exposure to radiation and oxidative agents. However, more work should be done on the mechanisms involved in damage and repair in these cells before drawing broader conclusions. A more expanded study involving repeated measurements across individual grasshoppers' ontogeny would help elucidate the nature of genetic damage accrual in this species, particularly whether it is consistent across time or whether it accelerates with more rapid development. Additionally, this would provide for a test of the hypothesis that insects with higher genetic damage as nymphs were at higher risk for early mortality.

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APPENDIX

Table A1: Comparison of fit statistics for the alternative general linear mixed models for the relationship between DNA damage (as a response variable, indexed by mean or 80th percentile DNA damage), parental radiation exposure, offspring sex, and developmental time (as measured by time to reach maturity)

	Mean DNA damage		80th percentile DNA damage	
Model	AICc	BIC	AICc	BIC
Full model: sex, time to maturity, (time to maturity)^2, radiation, sex × radiation, sex × time to maturity, sex × (time to maturity)^2	1,293.85	1,327.26	1,360.34	1,393.75
Sex, time to maturity, (time to maturity)^2, radiation, sex × time to maturity, sex × (time to maturity)^2	1,276.56	1,307.06	1,358.53	1,389.03
Sex, time to maturity, (time to maturity)^2, radiation, sex × radiation	1,289.60	1,317.17	1,356.05	1,383.62
Sex, time to maturity, (time to maturity)^2, radiation	1,274.96	1,299.57	1,354.17	1,378.77
Sex, time to maturity, (time to maturity)^2	1,274.67	1,296.29	**1,350.53**	1,372.14
Sex, time to maturity, radiation	1,333.79	1,355.41	1,382.11	1,403.72
Sex, time to maturity	1,331.18	1,349.78	1,379.51	1,398.11
Time to maturity, (time to maturity)^2	**1,270.69**	**1,289.29**	**1,351.31**	**1,369.91**
Time to maturity	1,335.75	1,351.31	1,382.57	1,398.13
Radiation	1,415.15	1,430.71	1,470.71	1,486.28
Sex	1,414.84	1,430.40	1,470.56	1,486.12
Null model	1,412.78	1,425.27	1,468.44	1,480.94

Note. All models included collection site and family (nested within collection site) as random effects. Fit statistics for the best-fit models are in bold. AICc, corrected Akaike information criterion; BIC, Bayesian information criterion.

Table A2: Comparison of fit statistics for the alternative general linear mixed models for the relationship between wing fluctuating asymmetry (as indexed by asymmetry in centroid size), mean DNA damage, and developmental time (as measured by time to reach maturity)

Model	AICc	BIC
Full model: sex, time to maturity, (time to maturity)^2, DNA damage, radiation, sex × time to maturity, sex × (time to maturity)^2, sex × DNA damage, sex × radiation	−845.77	−812.28
Sex, time to maturity, (time to maturity)^2, DNA damage, radiation	−859.04	−835.15
Sex, time to maturity, (time to maturity)^2, radiation	−859.94	−838.55
Sex, time to maturity, (time to maturity)^2, DNA damage	−847.14	−825.76
Sex, time to maturity, radiation	−849.04	−830.20
Sex, time to maturity, DNA damage	−849.24	−830.40
Sex, radiation, DNA damage	−865.79	−846.95
Time to maturity, radiation, DNA damage	−865.55	−846.71
Sex, time to maturity	−850.09	−833.83
DNA damage, radiation	**−867.38**	**−851.13**
Time to maturity, (time to maturity)^2	−850.54	−834.28
Sex	−852.32	−838.69
Time to maturity	−851.64	−838.00
Radiation[a]	**−867.56**	**−853.92**
DNA damage	−852.37	−838.73
Null model	−853.81	−842.83

Note. All models included collection site and family (nested within collection site) as random effects. Fit statistics for the best-fit models are in bold. Results were qualitatively similar if 80th percentile DNA damage was used instead of mean DNA damage (details not shown). AICc, corrected Akaike information criterion; BIC, Bayesian information criterion.

[a]The parameter estimation algorithm for this model did not converge. For this reason, we comment on the next best-fit model, which includes DNA damage and radiation. This latter model differs from the model including radiation alone by <2.0 for AIC and ≈2.0 for BIC and can therefore be considered equally informative as the best-fit model.

Literature Cited

Aghajanyan A., N. Kuzmina, A. Sipyagyna, L. Baleva, and I. Suskov. 2011. Analysis of genomic instability in the offspring of fathers exposed to low doses of ionizing radiation analysis of chromosome aberrations. Int J Radiat Biol 52: 538–546.

Almeida C., C. Pereira, T. Gomes, M.J. Bebianno, and A. Cravo. 2011. DNA damage as a biomarker of genotoxic contamination in *Mytilus galloprovincialis* from the south coast of Portugal. J Environ Monit 13:2559–2567.

Alonzo F., R. Gilbin, F.A. Zeman, and J.M. Garnier-Laplace. 2008. Increased effects of internal alpha irradiation in *Daphnia magna* after chronic exposure over three successive generations. Aquat Toxicol 87:146–156.

Barber R., M.A. Plumb, E. Boulton, I. Roux, and Y.E. Dubrova. 2002. Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. Proc Natl Acad Sci USA 99:6877–6882.

Barber R.C. and Y.E. Dubrova. 2006. The offspring of irradiated parents, are they stable? Mutat Res 598:50–60.

Beasley D.E., A. Bonisoli-Alquati, and T.A. Mousseau. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. Ecol Indic 30:218–226.

Beasley D.E., A. Bonisoli-Alquati, S.M. Welch, A.P. Møller, and T.A. Mousseau. 2012. Effects of parental radiation exposure on developmental instability in grasshoppers. J Evol Biol 25: 1149–1162.

Bonisoli-Alquati A., T.A. Mousseau, A.P. Møller, M. Caprioli, and N. Saino. 2010*a*. Increased oxidative stress in barn swallows from the Chernobyl region. Comp Biochem Physiol A 155:205–210.

Bonisoli-Alquati A., A. Voris, T.A. Mousseau, A.P. Møller, N. Saino, and M.D. Wyatt. 2010*b*. DNA damage in barn swallows (*Hirundo rustica*) from the Chernobyl region detected by use of the comet assay. Comp Biochem Physiol C 151:271–277.

Bookstein F. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge.

Carls N. and R.H. Schiestl. 1999. Effect of ionizing radiation on transgenerational appearance of pun reversions in mice. Carcinogenesis 20:2351–2354.

Carmona E.R., T.N. Guecheva, A. Creus, R. Marcos, and D. De Gene. 2011. Proposal of an in vivo comet assay using haemocytes of *Drosophila melanogaster*. Mutat Res 52:165–169.

Colinet H. and D. Renault. 2012. Metabolic effects of CO_2 anaesthesia in *Drosophila melanogaster*. Biol Lett 8:1050–1054.

DeBlock M. and R. Stoks. 2008. Compensatory growth and oxidative stress in a damselfly. Proc R Soc B 275:781–785.

Dizdaroglu M., P. Jaruga, M. Birincioglu, and H. Rodriguez. 2002. Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 32:1102–1115.

Dmitriew C.M. 2011. The evolution of growth trajectories: what limits growth rate? Biol Rev 86:97–116.

Dubrova Y.E. 2005. Radiation-induced mutation at tandem repeat DNA loci in the mouse germline: spectra and doubling doses. Radiat Res 163:200–207.

Dubrova Y.E., V.N. Nesterov, N.G. Krouchinsky, V.A. Ostapenko, R. Neumann, D.L. Neil, and A.J. Jeffreys. 1996. Human minisatellite mutation rate after the Chernobyl accident. Nature 280:683–686.

Dutilleul M., J.-M. Bonzom, C. Lecomte, B. Goussen, F. Daian, S. Galas, and D. Réale. 2014. Rapid evolutionary responses of life history traits to different experimentally-induced pollutions in *Caenorhabditis elegans*. BMC Evol Biol 14:252.

Dutilleul M., D. Réale, B. Goussen, C. Lecomte, S. Galas, and J.-M. Bonzom. 2017. Adaptation costs to constant and alternating polluted environments. Evol Appl 10:839–851, doi:10.1111/eva.12510.

Einor D., A. Bonisoli-Alquati, D. Costantini, T.A. Mousseau, and A.P. Møller. 2016. Ionizing radiation, antioxidant response and oxidative damage: a meta-analysis. Sci Total Environ 548/549: 463–471.

Furitsu K., H. Ryo, K.G. Yeliseeva, L. Thi, T. Thuy, H. Kawabata, E.V Krupnova, et al. 2005. Microsatellite mutations show no increases in the children of the Chernobyl liquidators. Mutat Res 581:69–82.

Galván I., A. Bonisoli-Alquati, S. Jenkinson, G. Ghanem, K. Wakamatsu, T.A. Mousseau, and A.P. Møller. 2014. Chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress in birds. Funct Ecol 28:1387–1403.

Garnier-Laplace J.M., K. Beaugelin-Seiller, C. Della-Vedova, J.-M. Métivier, C. Ritz, T.A. Mousseau, and A.P. Møller. 2015. Radiological dose reconstruction for birds reconciles outcomes of Fukushima with knowledge of dose-effect relationships. Sci Rep 5:16594.

Gotoh T. and K. Kikuchi. 2001. Rapid assessment of *Autographa californica* nuclear polyhedrosis virus infection of Sf-9 insect cells by analyzing suppression of reagent-induced apoptosis. Biotechnol Lett 23:1473–1478.

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

Ilnytskyy Y. and O. Kovalchuk. 2011. Non-targeted radiation effects–an epigenetic connection. Mutat Res 714:113–125.

Imamura T., S. Todoriki, N. Sota, H. Nakakita, H. Ikenaga, and T. Hayashi. 2004. Effect of "soft-electron" (low-energy electron) treatment on three stored-product insect pests. J Stored Prod Res 40:169–177.

Jirtle R.L. and M.K Skinner. 2007. Environmental epigenomics and disease susceptibility. Nature Rev Genet 8:253–262.

Klingenberg C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 11:353–357.

Klingenberg C.P., M. Barluenga, and A. Meyer. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56:1909–1920.

Klingenberg C.P. and G.S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52: 1363–1375.

Klingenberg C.P. and L.R. Monteiro. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst Biol 54:678–688.

Kovalchuk O. and J.E. Baulch. 2008. Epigenetic changes and nontargeted radiation effects—is there a link? Environ Mol Mutagen 49:16–25.

Langmaack M. and G. Schrader. 1997. Microhabitat analysis of three fen-grassland grasshopper species (Acrididae: Gomphocerinae). Entomol Gen 22:45–55.

Liang L., L. Deng, M.S. Mendonca, Y. Chen, B. Zheng, P.J. Stambrook, C. Shao, et al. 2007. X-rays induce distinct patterns of somatic mutation in fetal versus adult hematopoietic cells. DNA Repair 6:1380–1385.

Meehan K. 2004. Evaluation of DNA damage in a population of bats (*Chiroptera*) residing in an abandoned monazite mine. Mutat Res 557:183–190.

Møller A.P., J. Erritzøe, F. Karadas, and T.A. Mousseau. 2010. Historical mutation rates predict susceptibility to radiation in Chernobyl birds. J Evol Biol 23:2132–2142.

Møller A.P., A. Hagiwara, S. Matsui, S. Kasahara, K. Kawatsu, I. Nishiumi, H. Suzuki, et al. 2012. Abundance of birds in Fukushima as judged from Chernobyl. Environ Pollut 164:36–39.

Møller A.P., F. Karadas, and T.A. Mousseau. 2008. Antioxidants in eggs of great tits *Parus major* from Chernobyl and hatching success. J Comp Physiol B 178:735–743.

Møller A.P. and T.A. Mousseau. 2006. Biological consequences of Chernobyl: 20 years on. Trends Ecol Evol 21:200–207.

———. 2007. Species richness and abundance of forest birds in relation to radiation at Chernobyl. Biol Lett 3:483–486.

———. 2009. Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. Biol Lett 5:356–359.

———. 2013. Assessing effects of radiation on abundance of mammals and predator-prey interactions in Chernobyl using tracks in the snow. Ecol Indic 26:112–116.

———. 2015. Strong effects of ionizing radiation from Chernobyl on mutation rates. Sci Rep 5:8363.

Møller A.P., P.F. Surai, and T.A. Mousseau. 2005. Antioxidants, radiation and mutation as revealed by sperm abnormality in barn swallows from Chernobyl. Proc R Soc B 272:247–253.

Møller A.P. and J.P. Swaddle. 1997. Asymmetry, developmental stability and evolution. Oxford University Press, New York.

Monaghan P., N.B. Metcalfe, and R. Torres. 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Lett 12:75–92.

Monteiro V., D.G.S.M. Cavalcante, M.B.F. Viléla, S.H. Sofia, and C.B.R. Martinez. 2011. In vivo and in vitro exposures for the evaluation of the genotoxic effects of lead on the Neotropical freshwater fish *Prochilodus lineatus*. Aquat Toxicol 104:291–298.

Mousseau T.A. and C.W. Fox. 1998*a*. The adaptive significance of maternal effects. Trends Ecol Evol 13:403–407.

———, eds. 1998*b*. Maternal effects as adaptations. Oxford University Press, Oxford.

Mousseau T.A., T. Uller, E. Wapstra, and A.V. Badyaev. 2009. Evolution of maternal effects: past and present. Philos Trans R Soc B 364:1035–1038.

Natarajan A.T. 2006. Induced transgenerational genetic effects in rodents and humans. J Radiat Res 47:B39–B43.

Nomura T. 1982. Parental exposure to X rays and chemicals induces heritable tumours and anomalies in mice. Nature 296:575–577.

———. 1990. Of mice and men? Nature 345:671.

———. 2006. Transgenerational effects of radiation and chemicals in mice and humans. J Radiat Res 47:B83–B97.

Ozturk S. and N. Demir. 2011. DNA repair mechanisms in mammalian germ cells. Histol Histopathol 26:505–517.

Parisot F., J.-P. Bourdineaud, D. Plaire, C. Adam-Guillermin, and F. Alonzo. 2015. DNA alterations and effects on growth and reproduction in *Daphnia magna* during chronic exposure to gamma radiation over three successive generations. Aquat Toxicol 163:27–36.

Perera F. and J. Herbstman. 2011. Prenatal environmental exposures, epigenetics, and disease. Reprod Toxicol 31:363–373.

Rigonato J., M. Mantovani, and B. Jordao. 2005. Comet assay comparison of different *Corbicula fluminea* (Mollusca) tissues for the detection of genotoxicity. Genet Mol Biol 28:464–468.

Rohlf F.J. 2005. tpsDig, digitize landmarks and outlines. State University of New York, Stony Brook.

Schilling-Tóth B., N. Sándor, E. Kis, M. Kadhim, G. Sáfrány, and H. Hegyesi. 2011. Analysis of the common deletions in the mitochondrial DNA is a sensitive biomarker detecting direct and non-targeted cellular effects of low dose ionizing radiation. Mutat Res 716:33–39.

Schindewolf C., K. Lobenwein, K. Trinczek, M. Gomolka, D. Soewarto, W. Pargent, N. Singh, et al. 2000. Comet assay as a tool to screen for mouse models with inherited radiation sensitivity. Mamm Genome 11:552–554.

Shestopalov V.M. 1996. Atlas of Chernobyl Exclusion Zone. Ukrainian Academy of Science, Kiev.

Singh N.P., M.T. McCoy, R.R. Tice, and E.L. Schneider. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191.

Skinner M.K., M. Manikkam, and C. Guerrero-Bosagna. 2010. Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab 21:214–222.

Stoks R. and M. DeBlock. 2011. Rapid growth reduces cold resistance: evidence from latitudinal variation in growth rate, cold resistance and stress proteins. PLoS One 6:e16935.

Stoks R., M. DeBlock, and M.A. McPeek. 2006. Physiological costs of compensatory growth in a damselfly. Ecology 87: 1566–1574.

Sykes P.J., A.A. Morley, and A.M. Hooker. 2006. The PKZ1 recombination mutation assay: a sensitive assay for low dose studies. Dose-Response 4:91–105.

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

Tice R.R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, and Y.F. Sasaki. 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221.

Todoriki S., M. Hasan, A. Miyanoshita, T. Imamura, and T. Hayashi. 2006. Assessment of electron beam-induced DNA damage in larvae of chestnut weevil, *Curculio sikkimensis* (Heller) (Coleoptera: Curculionidae) using comet assay. Radiat Phys Chem 75:292–296.

TriTek. 2003. Comet score. TriTek, Sumerduck, VA.

Vedenina V.Y. and O. Helversen. 2003. Complex courtship in a bimodal grasshopper hybrid zone. Behav Ecol Sociobiol 54:44–54.

Walters R.J., M. Hassall, M.G. Telfer, G.M. Hewitt, and J.P. Palutikof. 2006. Modelling dispersal of a temperate insect in a changing climate. Proc R Soc B 273:2017–2023.

Ward J.F. 1988. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol 35:92–125.

Weinberg H.S., A.B. Korol, V.M. Kirzhner, A. Avivi, T. Fahima, E. Nevo, S. Shapiro, et al. 2001. Very high mutation rate in offspring of Chernobyl accident liquidators. Proc R Soc B 268:1001–1005.

Zainullin V.G., V.A. Shevchenko, E.N. Mjasnjankina, M.V. Generalova, and A.O. Rakin. 1992. The mutation frequency of *Drosophila melanogaster* populations living under conditions of increased background radiation due to the Chernobyl accident. Sci Total Environ 112:37–44.

Zeng G., T.K. Day, A.M. Hooker, B.J. Blyth, M. Bhat, W.D. Tilley, and P.J. Sykes. 2006. Non-linear chromosomal inversion response in prostate after low dose X-radiation exposure. Mutat Res 602:65–73.