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GLOBAL CYTOSINE METHYLATION IN *DAPHNIA MAGNA* DEPENDS ON GENOTYPE, ENVIRONMENT, AND THEIR INTERACTION

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Abstract: The authors characterized global cytosine methylation levels in 2 different genotypes of the ecotoxicological model organism *Daphnia magna* after exposure to a wide array of biotic and abiotic environmental stressors. The present study aimed to improve the authors' understanding of the role of cytosine methylation in the organism's response to environmental conditions. The authors observed a significant genotype effect, an environment effect, and a genotype × environment effect. In particular, global cytosine methylation levels were significantly altered after exposure to *Triops* predation cues, *Microcystis*, and sodium chloride compared with control conditions. Significant differences between the 2 genotypes were observed when animals were exposed to *Triops* predation cues, *Microcystis*, *Cryptomonas*, and sodium chloride. Despite the low global methylation rate under control conditions (0.49–0.52%), global cytosine methylation levels upon exposure to *Triops* demonstrated a 5-fold difference between the genotypes (0.21% vs 1.02%). No effects were found in response to arsenic, cadmium, fish, lead, pH of 5.5, pH of 8, temperature, hypoxia, and white fat cell disease. The authors' results point to the potential role of epigenetic effects under changing environmental conditions such as predation (i.e., *Triops*), diet (i.e., *Cryptomonas* and *Microcystis*), and salinity. The results of the present study indicate that, despite global cytosine methylation levels being low, epigenetic effects may be important in environmental studies on *Daphnia*. *Environ Toxicol Chem* 2015;9999:1–6. © 2015 SETAC

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INTRODUCTION

Understanding how organisms respond to environmental changes is a key question in ecology and environmental toxicology. Recent developments in molecular technologies and analytical methods have resulted in an increased understanding of how organisms respond to their environment [1,2]. One of the mechanisms that may mediate responses to environmental change involves epigenetic modification, but this is currently underexplored in nonmammalian model organisms. To date, little attention has been given to epigenetic modifications in ecology and environmental toxicology [3,4]. Well-known epigenetic modifications are changes in cytosine methylation or histone modifications resulting in DNA modifications without altering the underlying sequence. These epigenetic changes are of particular interest not only because they are affected by environmental changes but also because of their heritability. Epigenetic changes may be passed on to offspring even after the initial stress has disappeared [4–6].

The interaction between epigenetic mechanisms and the environment is particularly well studied in mammals [7]. Studies with mammalian model organisms have revealed epigenetic changes in response to environmental factors such as malnutrition and toxicants as well as their potential role in disease development [8–10]. Baccarelli and Bollati [11] discuss epigenetic effects on mammalian model organisms invoked by a wide array of chemicals, most of which are known persistent environmental pollutants in many ecosystems such as arsenic, methylmercury, and persistent organic pollutants. Yet, only few studies have focused on epigenetic effects in ecotoxicological model organisms such as the invertebrate *Daphnia*. In addition to being a standard ecotoxicological model, *Daphnia* has been put forward as a potential epigenetic model [12]. The parthenogenetic reproduction cycle of *Daphnia* allows researchers to study epigenetic variation in the absence of genetic variation. Furthermore, the large body of ecotoxicological literature and the availability of its genome sequence make *Daphnia* an ideal candidate to study epigenetic effects in an ecological or ecotoxicological context [12]. Vandegehuchte et al. [13] observed changes in global cytosine methylation levels of *D. magna* upon exposure to chemicals known to affect DNA methylation in other organisms. Vandegehuchte et al. [14,15] also studied changes in global cytosine methylation upon exposure of *D. magna* to cadmium and zinc and observed changes after exposure to zinc but not cadmium. Studies of DNA methylation in other invertebrates have primarily been functional studies in a variety of organisms, including honeybee, silkworm, ant, pacific oyster, sea urchin, and sea squirt [16]. These studies demonstrate that, despite their relatively low global cytosine methylation level compared with vertebrates (0.22%–0.90% vs 5%–10% [17]), invertebrate species do experience changes in cytosine methylation as a result of changes in environmental conditions.

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To further explore genotype × environment interactions and the environmental specificity of epigenetic control in *D. magna*, we characterized the changes in global cytosine methylation in 2 different genotypes of *D. magna* after exposure to a wide variety of different environments caused by both natural and anthropogenic factors. We tested the hypothesis that changes in global cytosine methylation depend on the genotype, the environment, and genotype × environment interactions. Daphnids can thrive in a wide variety of environmental conditions and have a large phenotypic plasticity [18]. Many of these biological responses to changes in environmental conditions have been described extensively in the literature [19]. Phenotypic responses to predation or parasitism have, for example, been described by, among others, De Meester [20], Decaestecker et al. [21], and Petrusek et al. [22], and phenotypic metal stress responses have been described by Shaw et al. [23] and Asselman et al. [24]. In the present study, we explored the extent to which exposure to different environments can lead to epigenetic changes in *Daphnia*.

We exposed *D. magna* to 15 different environments, probing for a large variety of environmental changes to which *Daphnia* can be exposed. Three heavy metals were selected to represent metal pollution: arsenic, cadmium, and lead. We also exposed *Daphnia* to 4 types of food that widely varied in quality: normal-quality food (a mixture of *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii* representing the commonly used algae in ecotoxicological studies [25]), excellent food quality (the unicellular alga *Cryptomonas*), nontoxic low-quality food (non–microcystin-producing *Microcystis* [26]), and toxic low-quality food (microcystin-producing *Microcystis*). In terms of stress caused by biotic factors, we exposed *Daphnia* to predation (predator cues of fish and *Triops*) and parasitism (exposure to spores of the bacterium causing white fat cell disease). Finally, we studied the response upon changes in water quality such as changes in pH, salinity (NaCl), temperature, and oxygen concentration. Although the metals are typical test chemicals in ecotoxicology, the other environmental conditions reflect factors that may influence the susceptibility to pollutants and sometimes synergistically interact with pollutants [27,28].

MATERIALS AND METHODS

Experimental design

Experiments were conducted with 2 different genotypes of *D. magna*, hereafter referred to as Iinb1 and Xinb3. Both genotypes were obtained from The Ebert Group (Universität Basel, Basel, Switzerland). The genotypes were selected because they have been used to produce the genetic linkage map of *D. magna* (with associated mapping panel) and because the Xinb3 genotype is also used in the ongoing genome sequencing project to produce a reference genome assembly [29]. It was anticipated that this selection would significantly facilitate future mechanistic research based on the results reported in the present study. The Xinb3 genotype is an inbred clonal lineage that was established by 3 generations of selfing that started with a clonal lineage isolated from Tvärminne, Finland, whereas the Iinb1 was established by 1 generation of selfing with a clone that originates from Munich, Germany [29]. Both clonal lineages were then reared isoclonally in The Ebert Group for at least 5 yr (at least 100 generations) and further in our own laboratory for another 2 yr (at least 50 generations) in identical laboratory conditions prior to experiments. While this long history post isolation from their original environment should exclude that any observed differences in the short-term effects of environmental changes on global cytosine methylation between the 2 genotypes would be related to any differences in their ancestral environments, this can never be guaranteed completely. Therefore, the "genotype effects" and "genotype × environment" effects on global methylation levels reported in the present study can be the result of both genetic differences (i.e., DNA sequence) and epigenetic differences at the individual cytosine level (e.g., methylation status) between the 2 clones.

Experiments were conducted in ADaM medium [30] in a climate-controlled chamber ($20 \pm 1\ ^{\circ}C$) under a 16:8-h light: dark cycle. At least 2 generations of both genotypes were grown from birth to reproducing female under those experimental conditions prior to the stress exposures, and neonates from the third clutch of these (second or further generation) mothers were used in the exposures. Neonates were grown at a density of 20 daphnids per liter until day 14, after which they were assigned to a specific treatment and exposed for 48 h. After 48 h, experiments were taken down and animals were immediately flash-frozen in liquid nitrogen and stored at $-80\ ^{\circ}C$ until further analysis. All animals, neonates and brood mothers, were fed daily with an algal mixture consisting of *P. subcapitata* and *C. reinhardtii* in a 3:1 mixture ratio based on cell numbers, reflecting normal food quality. Final feeding concentration was 0.15 mg carbon per daphnid. Medium was renewed every 2 d until day 14, which was the start of the exposure.

For each treatment, 3 different biological replicates were set up per genotype (i.e., 3 different glass jars of 500 mL). Each jar contained 10 individual daphnids. The concentrations in the 3 metal treatments and the salinity treatment are reported in Table 1. Stock solutions were added to the medium at the start of the exposure to achieve the final concentration reported in Table 1. The cadmium concentration was selected based on previous experiments by De Coninck et al. [31] and Muyssen et al. [32]. Arsenic, lead, and sodium chloride concentrations were selected based on acute 50% lethal concentration values reported in Biesinger and Christensen [33]. In treatments with *Cryptomonas* (SAG 26.80, Culture Collection of Algae, University of Göttingen) or *Microcystis* (microcystin producer PCC7806, Pasteur Culture Collection; non–microcystin producer CCAP 1450/1, Culture Collection of Algae and Protozoa), the diet of the animals was modified as 50% of the above-mentioned (feeding) algal mixture was replaced with *Cryptomonas* or *Microcystis*, respectively. These ratios were selected based on previous results with cyanobacteria from Hochmuth et al. [34]. The temperature treatment was conducted in a separate climate-controlled chamber at $28\ ^{\circ}C$ under the same light regime. In the pH treatments, the pH was reduced or increased with HCl or NaOH, respectively, until the targeted pH was reached. This was verified using a pH meter (P407; Consort). The pH glass electrode was calibrated before each use using pH buffers of 4 and 7 (Merck).

Predation treatments consisted of exposing animals to predation cues from fish or *Triops* using fish- or *Triops*-conditioned medium. For the fish-conditioned medium, we filtered water (Millipore filter, mesh size 0.45 μm) drawn from a 20-L aquarium in which 19 three-spined sticklebacks (*Gasterosteus aculeatus*) with a standard length of 6 cm had been kept for 24 h. This medium was diluted 5 times with ADaM medium to obtain a final amount of predation cues from 19 sticklebacks in 100 L. Sticklebacks were fed living *Daphnia* during the experiment but in a separate container to avoid *Daphnia* cues in the fish-conditioned medium; that is, fish were removed from the medium to a separate container for feeding and placed back into

Table 1. Summary of all studied environmental changes, their ecological context, and the specifications of the exposure condition or treatment

Environmental change	Type	Ecological context	Exposure condition
Pollution			
Arsenic ($NaAsO_2$)	Abiotic	Heavy metal	1000 μg/L
Cadmium ($CdCl_2$)	Abiotic	Heavy metal	6 μg/L
Lead ($PbCl_2$)	Abiotic	Heavy metal	166 μg/L
Water quality			
Sodium chloride	Abiotic	Salinity	5 g/L
Hypoxia	Abiotic	Decreased oxygen concentration	3 mg O_2/L
pH 5.5	Abiotic	Acidification	pH 5.5
pH 8	Abiotic	Alkaline water	pH 8
Temperature	Abiotic	Increased temperature	28 °C
Food type			
Pseudokirchneriella, Chlamydomonas	Biotic	Normal-quality food (control)	100% of diet
Cryptomonas	Biotic	High-quality food, high polyunsaturated fatty acid content	50% of diet
Microcystis (toxic)	Biotic	Low-quality food, toxin producer	50% of diet
Microcystis (nontoxic)	Biotic	Low-quality food	50% of diet
Biotic factors			
Fish	Biotic	Predation cue (kairomones)	19 sticklebacks in 100 L fish condition medium
Triops	Biotic	Predation cue (kairomones)	1 *Triops* in 2 L
White fat cell disease	Biotic	Parasite infection	Solution of 5 infected animals/d

the medium. For the *Triops*-conditioned medium, we cultured 1 adult *Triops*, with a standard length of approximately 3 cm, in a total volume of 2 L ADaM for 24 h [35]. *Triops* were fed living *Daphnia* but in a separate container to avoid *Daphnia* cues in the *Triops*-conditioned medium; that is, fish were removed from the medium to a separate container for feeding and placed back into the medium. Prior to use, this medium was filtered using a Millipore filter, mesh size 0.45 μm.

The hypoxia treatment was set up in Winkler bottles of 250 mL (VWR), each containing 5 daphnids, to maintain the same density as in the other treatments. Oxygen levels in these treatments were fixed at 3 mg/L by aerating the water with N_2 gas while simultaneously monitoring the oxygen concentration with an oxygen electrode (WTW330; Wissenschaftlich Technische Werkstätten GmbH) until the required oxygen level was obtained. To exclude any potential effect of the Winkler bottles, an additional control treatment of 3 replicates was set up in Winkler bottles under normal oxygen conditions (i.e., 9 mg/L).

The parasite treatment consisted of exposing the daphnids to a solution containing spores of the microorganism that causes white fat cell disease in *Daphnia* following the protocol of Decaestecker et al. [36]. To exclude effects of adding the spore solution itself (which also contains homogenized *Daphnia* tissue), a control treatment of 3 replicates was set up, which received a placebo solution. The white fat cell disease solution was made by crushing white fat cell disease–infected *Daphnia*. Crushing the infected animals results in setting free the spores, after which the spores are suspended. To standardize exposure conditions, we created spore solutions corresponding to a density of 1 infected animal per milliliter (e.g., crushed 15 infected *Daphnia* to generate 15 mL of spore solution). This procedure is analogous to, albeit a bit milder than, that of Decaestecker et al. [36] to exclude mortality of the organisms and should nevertheless lead to a relatively high infectivity. Parasite infection with white fat cell disease occurs through horizontal transfer of a dead host [36]. The placebo solution was made in exactly the same manner but by crushing noninfected *Daphnia* and suspending the homogenized tissue at a concentration of 1 noninfected animal per milliliter. In the parasite treatment, 5 mL of the white fat cell disease solution was added to each jar at the start of the exposure and after 24 h. When adding the solution, jars were gently stirred to allow optimal mixing. The corresponding control treatment received 5 mL of the placebo solution at the start of the exposure and after 24 h.

DNA extraction, digestion, and hydrolysis

We extracted DNA from frozen tissue using the Masterpure™ kit (Epicentre) following the manufacturer's instructions. The quality and quantity of DNA were verified using a Nanodrop 2000 spectrophotometer (Nanodrop Products). Quality criteria consisted of a 260/280 ratio between 1.8 and 2.0. Subsequent hydrolysis of DNA was performed according to Quinlivan and Gregory [37]. Briefly, DNA hydrolyzed to nucleosides by adding a digestion mix containing benzonase nuclease, phosphodiesterase, and alkaline phosphatase and incubating the samples with this digestion mix at 37 °C for 6 h.

Quantification of global cytosine methylation level

The global DNA methylation level of the hydrolyzed DNA samples was detected and quantified using an Accela ultrahigh performance liquid chromatography system (Thermo Fisher Scientific) coupled to a TSQ Vantage Triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific) by means of a heated electrospray ionization interface.

Liquid chromatographic separation of the nucleosides occurred on a Waters Acquity UPLC HSS T3 1.8 μm column (2.1 mm × 100 mm) at a flow rate of 300 μL/min. A binary solvent system was used: 0.08% formic acid in water and 0.08% formic acid in acetonitrile. The stepwise linear gradient started from 99% solution A and went up to 90% solution B in 2.5 min, after which the column was allowed to re-equilibrate for 3.5 min, characterized with a total run time of 9 min. For the mass spectrometric method, the following work conditions were applied: operation in positive ionization mode; capillary and vaporizer temperature at 305 °C and 200 °C, respectively; sheath and auxiliary gas at 35 arbitrary units and 5 arbitrary units, respectively; spray voltage of 5 kV; and discharge current of 4 μA. Mass/charge ratios of detected ions for 5-methyl-2′-deoxycytidine (mdC) were 242.076 (precursor ion) > 126.032, 109.012 (product ions); for deoxyguanosine (dG) these were 268.058 > 134.983, 152.021. These product ions were used for the quantification of [mdC] and [dG] in the digests, using externally prepared standard series with commercially available mdC (US Biological) and dG (Aldrich). The global DNA

methylation level is expressed as the relative mdC (percent mdC) content, that is, as percent [mdC]/[dG] or percent [mdC]/([dC] + [mdC]) [38].

Statistical analysis

The global DNA methylation levels, expressed as percent [mdC], were analyzed using a 2-way analysis of variance (ANOVA) with 2 factors, environment (the different treatments) and genotype, in Statistica version 7.0 (Statistica). For post hoc comparisons, p values were corrected for multiple testing using Duncan's test. To adhere to the requirements of ANOVA (i.e., normality and homoscedasticity of the data), the ANOVA was conducted on rank-transformed data.

RESULTS AND DISCUSSION

Full factorial ANOVA indicated a significant effect of genotype ($p < 0.001$), of environment ($p < 0.001$), and of the genotype × environment interaction ($p < 0.001$). The significant main effect of genotype observed in the ANOVA reflects that, overall, clone Xinb3 has a higher DNA methylation level than clone Iinb1. The significant genotype × environment interaction effect reflects that this difference between the 2 genotypes is dependent on the environmental condition. Global DNA methylation levels were not significantly different between genotypes under control conditions ($p = 0.84$; Supplemental Data, Table S1). In the Iinb1 genotype, global DNA methylation was $0.49 \pm 0.19\%$ on average, whereas the Xinb3 genotype had a global methylation level of $0.52 \pm 0.16\%$. Furthermore, global DNA methylation levels in controls specifically designed to exclude potential effects related to the treatments of hypoxia and exposure to spores of white fat cell disease did not differ significantly between genotypes (hypoxia, $p = 0.34$; white fat cell disease, $p = 0.94$; Supplemental Data, Tables S1 and S3) or from the control treatment (hypoxia control, $p = 0.38$; white fat cell disease control, $p = 0.81$; Supplemental Data, Tables S2 and S3). Global DNA methylation levels reported in the present study (i.e., 0.49–0.52%) under control conditions are in line with those reported by Vandegehuchte et al. [15], who reported DNA global methylation levels between 0.25% and 0.40% in a *D. magna* genotype identified as K6, isolated from a pond in Antwerp (Belgium). In addition, they are in line with the low global DNA methylation levels reported in other arthropods (e.g., 0.11% in *Bombyx mori*, 0.7% in Acyrtosiphon pisum, 0.11–0.15% in ant species) [39].

Three environmental conditions differed significantly in their global DNA methylation pattern from the control treatment. First, a significant decrease in global DNA methylation was observed for the Iinb1 genotype exposed to non–microcystin-producing *Microcystis*. Second, global DNA methylation was significantly lower when the Iinb1 genotype was exposed to sodium chloride compared with control conditions. Third, exposure to *Triops* predation cues significantly affected global DNA methylation levels in both genotypes compared with control conditions (Figure 1; Supplemental Data, Tables S1 and S3). Mean global DNA methylation levels in the Iinb1 genotype were 0.23%, 0.21%, and 0.21% for exposure to non–microcystin-producing *Microcystis*, sodium chloride, and *Triops*, respectively. In contrast, mean global DNA methylation levels in the Xinb3 genotype significantly increased when exposed to *Triops*, which resulted in a mean global DNA methylation level of 1.02%, whereas no significant differences were observed when the Xinb3 genotype was exposed to sodium chloride or non–microcystin-producing *Microcystis*. Although a main cadmium effect was observed (Supplemental Data, Table S2; $p = 0.04$), this effect was not present when comparing the methylation patterns of control and exposed animals within each

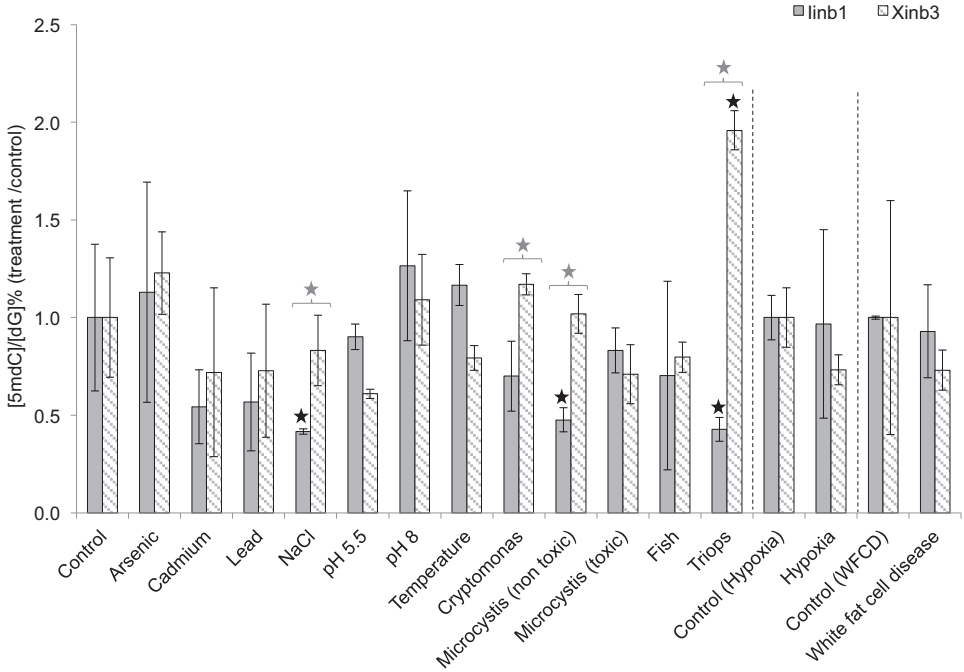

Figure 1. Mean global DNA methylation level in all treatments for the 2 genotypes Iinb1 and Xinb3 normalized to the mean corresponding control methylation (i.e. control for all treatments excluding hypoxia and white fat cell disease [WFCD], which each have their respective controls). Error bars represent standard deviation. Black stars denote exposures significantly different from the corresponding control treatment ($p < 0.05$). Gray stars denote exposures for which Iinb3 and Xinb3 differ significantly from one another ($p < 0.05$).

genotype ($p_{\text{Iinb1}} = 0.09$, $p_{\text{Xinb3}} = 0.30$; Supplemental Data, Table S1).

Global DNA methylation levels differed significantly between the 2 genotypes when exposed to *Cryptomonas*, non–microcystin-producing *Microcystis*, sodium chloride, and *Triops*. In these 4 exposures, global DNA methylation levels were significantly higher in the Xinb3 genotype than in the Iinb1 genotype, varying by at least a factor 2, up to even a factor 5 (*Triops* treatment; Figure 1; Supplemental Data, Tables S1 and S3). The between-replicate variance of global cytosine methylation differed between treatments and between genotypes (Levene test, $p < 0.05$). This may be explained by differences in phenotypic parameters affecting the DNA extraction and DNA methylation detection (e.g., body weight and size of the organism) as well as by true differences in variance in global methylation levels. At present, the exact sources of these differences in variance remain unclear but are worthy of further research.

These results highlight that, despite the overall low global DNA methylation in *Daphnia*, DNA methylation levels may vary with at least a factor of 5 between the responses to changes in environmental conditions. The number of exposures causing a significant effect on global DNA methylation is smaller than expected based on the literature. Indeed, the majority of the environmental conditions tested in the present study have been reported to cause changes in DNA methylation patterns in at least 1 other species [39]. Changes in DNA methylation in response to higher temperature have, for example, been reported in the sea bass, and altered DNA methylation patterns have been observed among others in rice species as well as earthworm species upon exposure to metals [40–42]. There are a number of possible explanations for the low number of significant responses. First, our approach only detects changes in global DNA methylation levels (i.e., total fraction of methylated cytosines relative to the total measured guanines or cytosines). As a result, changes in methylation patterns at the level of genes and cytosine positions that do not alter the global DNA methylation level can therefore not be observed. Second, we quantified changes in DNA methylation level only in the generation that was exposed to the stressor; the response to some stressors might be greater in the offspring generation. This was observed by Vandegehuchte et al. [15] when they exposed *D. magna* to zinc concentrations, observing significant changes in global DNA methylation level only in the F1 (offspring) generation and not in the parental generation. Finally, differences in culture conditions, exposure concentrations, and duration may also lead to different results among studies [43].

We also observed differences between environmental changes with the same ecological context. For example, exposure to predation cues resulted in changes in global DNA methylation levels only when the predator was *Triops*; no changes were observed when fish were used as predators. Different food types resulted in different global DNA methylation patterns (Figure 1). Exposure to low-quality food resulted in a significant decrease in global DNA methylation for the Iinb3 genotype compared with standard-quality food. However, no significant differences were observed between standard-quality food and toxic low-quality food (Figure 1). These results are in line with known epigenetic effects of nutrition in other models such as honeybees and mammals [44,45]. Furthermore, the results highlight that different genotypes respond differently to nutritional changes in terms of their global DNA methylation pattern. These differences do not depend on the food quality, as the same pattern was observed for both food types. Indeed, exposure to both high-quality (*Cryptomonas*) and low-quality (non–microcystin-producing *Microcystis*) food types resulted in significantly higher global DNA methylation in the Xinb3 genotype than in the Iinb1 genotype.

Only 1 abiotic stressor (i.e., sodium chloride or salt stress) significantly affected global DNA methylation levels. Changes in DNA methylation patterns in response to salt stress have been reported in plants [46,47]. Although epigenetic regulation has been suggested as a mechanism for phenotypic plasticity in environments with changed salinity, no experimental evidence in invertebrates has been reported so far [48].

The results of our assessment of epigenetic responses to a broad array of environmental conditions clearly illustrate that changes in global DNA methylation are the result of a complex interaction between genotype and environment. The 2 genotypes did not differ in their global DNA methylation levels under control, unstressed conditions; but significant differences did appear after exposure to 4 specific environmental conditions. Reports of genotype–environment interactions in DNA methylation are rare, and we found only 2 studies in invertebrates. Snell-Rood et al. [49] observed a significant diet × genotype interaction on cytosine methylation in the beetle *Onthophagus* sp., and Kille et al. [40] studied arsenic tolerance in 2 lineages of the earthworm *Lumbricus rubellus* and observed a clear association between arsenic tolerance and cytosine methylation in 1 of the 2 lineages but not in the other.

The present results have some important implications for current practices in ecotoxicology. First, effects of changes in environmental conditions on DNA methylation levels cannot be excluded, and the potential for transgenerational effects on cytosine methylation needs further study. Second, variation in variables that are often considered to be less important or even ignored in experiments, such as changes in food types and presence of predators, may have significant effects on DNA methylation levels, which in turn may have an impact on the fitness of the organism. Third, genotype × environment interactions are not limited to phenotypic responses but are also present at the epigenetic level.

CONCLUSION

We report changes in global cytosine methylation levels of 2 *Daphnia magna* genotypes after exposure to a wide array of environmental stressors. Significant differences were observed in exposures with sodium chloride, *Triops* predation cues, and diets containing *Cryptomonas* or non–microcystin-producing *Microcystis*. Although no significant differences in global DNA methylation levels were observed between the 2 genotypes under control conditions, significant differences between genotypes were observed with exposure to some of the environmental stressors tested, indicating a genotype × environment effect. The results of the present study indicate that epigenetic effects may be important in environmental studies on *Daphnia*, even though overall methylation levels are low.

SUPPLEMENTAL DATA

Tables S1–S3. (1.6 MB XLS).

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